STUDIES ON THE LATERAL LINE SYSTEM OF THE AFRICAN CLAWED TOAD AT LARVAL AND ADULT STAGES OF DEVELOPMENT

by

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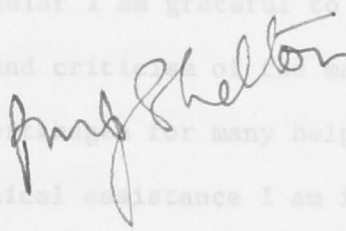
DECLARATION

I declare that the work reported in this thesis is my own and has not been previously submitted for any other degree. Equations describing surface wave properties were derived by J.W.G. Giddings Esq. and Dr. S.M.A. Meggitt of The Australian National University.



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SUMMARY

1. This thesis describes the lateral line system in *Xenopus laevis* tadpoles and examines changes in the system which occur at metamorphosis. The findings are discussed in relation to the known development of other sensory systems.
2. The lateral line system is well developed in tadpoles prior to metamorphosis and the morphological polarization of receptor cells, within single organs, provides anatomical evidence for their directional sensitivity. Lateral line nerve trunks contain both myelinated and unmyelinated nerve fibres in the tadpole. It is likely that myelinated fibres have an afferent function. Two types of nerve ending on the hair cells probably have afferent and efferent functions. A third type of process may represent another class of nerve terminal.
3. Each larval plaque is innervated by two spontaneously active afferent units. Their firing rates are changed by mechanical displacement of the receptor cell hair processes and they show directional sensitivity to water currents. Efferent units are not spontaneously active but fire during gill and tail movements. Stimulation of the lateral line system does not alter the patterns of efferent activity.

4. Efferent lateral line fibres are unmyelinated in the tadpole, they become myelinated at metamorphosis. There is an increase in the conduction velocities of efferent units at this stage and ipsilateral and contralateral stimulation of the lateral line rows become effective stimuli for increasing efferent activity.
5. There is a peripheral reorganization of the lateral line rows at metamorphosis, including positional changes in organ distribution and a reduction in the total number of organs. Rearrangement is particularly noticeable in the supra-orbital row and this reflects its special adult function in the detection of surface waves.
6. Hair cell innervation has been examined in an adult lateral line organ. All receptor cells with the same morphological polarization are innervated by branches of the same afferent nerve fibre. This explains the dual sensory innervation of a single plaque. Efferent endings are the terminals of thin myelinated fibres. They make synaptic contact with both the afferent endings and the hair cells.

CHAPTER I

INTRODUCTION

Metamorphosis, the change from a free living juvenile form to an adult with radically different morphology and mode of life, provides excellent material for the study of adaptive modification of the nervous system during development. The amphibians and holometabolous insects show particularly striking changes at metamorphosis (Fig. 1) but the two groups achieve these changes in rather different ways. In holometabolous insects larval and adult nervous systems develop independantly from an early time in embryogenesis. In the Amphibia, on the other hand, the fundamental organization of the central nervous system is established in the larva; the adult nervous system develops from this by addition of new elements and the refinement of existing pathways.

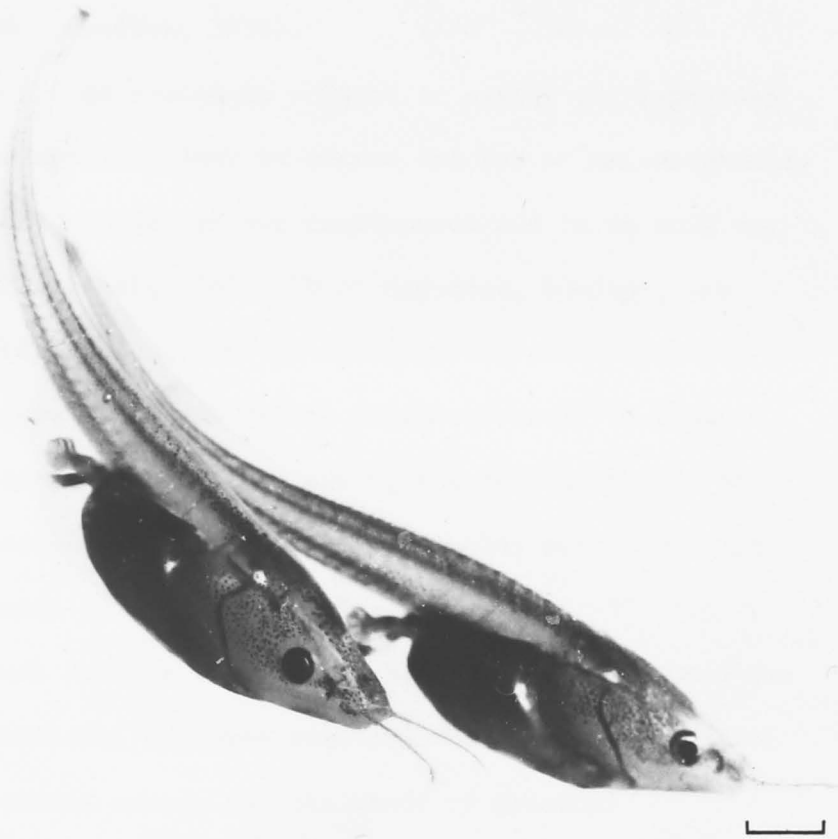
The unique feature of amphibian metamorphosis is that the larva is active during the time when the major changes in functional organization of the nervous system take place. Changes in body form, habitat and diet are necessarily accompanied by changing patterns of sensory input. Examples of sensory systems which have been studied at this transitional period include the development of binocular vision in the frog (Gaze, Keating, Székely and Beazley, 1970; Jacobson, 1971), increasing complexity of retinal organization (Pomeranz and Chung, 1970), the growth of new connections in amphibian

Figure 1.

These photographs show the marked difference in overall anatomy of the tadpole and adult stages of development. The larva maintains a "head-down" posture and uses its tail for locomotion. It is a filter feeder and continually undulates the tail tip. The adult has the usual frog-like appearance and breathes at the water surface. It is somewhat unusual in that it is aquatic for all of its life. It feeds on insects, worms and other invertebrates.

Scale upper 0.5 cm.

lower 2.0 cm.



cutaneous innervation (Miner, 1956; Jacobson and Baker, 1968, 1969), the redistribution of lateral line organs in *Xenopus* (Paterson, 1939; Shelton, 1970).

The lateral line system is present in nearly all amphibian tadpoles and is normally lost in anuran species at metamorphosis. Where it persists the adults are carnivorous and it is used in prey capture (Dijkgraaf, 1967). Most tadpoles, however, are vegetarians (Wright, 1951) and so the function of the lateral line system may be different before metamorphosis. In those species where it persists in the adult, the redistribution of organs at metamorphosis argues against it being merely a stage in the development of the adult system.

Because such obvious functional changes occur and since the organs are superficial and have simple innervation, the lateral line is a favourable system for the study of neuronal reorganization at metamorphosis. This study sets out to demonstrate the structure and function of the system in larval *Xenopus laevis* (Daudin) and describes in detail the changes which take place as the system develops into that of the adult.

I. The development of nervous systems

It is an indication of our poor understanding of neural ontogeny that several fundamentally different approaches which have been used to study the subject still remain separate. Emerging behaviour patterns can be related to changes in anatomical and physiological organization (Coghill, 1914, 1929; Herrick and Coghill, 1915; Youngstrom, 1938; Hooker, 1952; Whiting, 1955; Hughes and Prestige, 1967; Riss, 1969; Bentley and

Hoy, 1970) or neuronal development can be studied within the context of developmental mechanics (Detwiler, 1936; Piatt, 1948; Glücksmann, 1951; Hamburger, 1952; Hughes, 1968a). A third and more recent approach is to determine how precise patterns of neural connections are specified (Sperry, 1963; Székely, 1966; Jacobson, 1969; Gaze, 1970). Some examples of studies in each of these fields are outlined below to give an indication of the value of different approaches.

(i) Behaviour related to anatomy and physiology

The classical concept of the sequential development of the vertebrate nervous system (Coghill, 1929) favours the view that changes in behaviour are reflected in additions to and refinements of existing channels in the nervous system rather than the growth of entirely new systems. This view arose from Coghill's study on the early development of *Ambystoma* in which central anatomy was correlated with the development of spinal reflexes.

Prior to the growth of the dorsal root ganglia the afferent system of the spinal cord of lower vertebrates consists of a chain of giant sensory cells (Coghill, 1914), known as the Rohon-Beard cells (Rohon, 1885; Beard, 1889, 1892, 1896) which are located in the dorsal part of the spinal cord. On the basis of correlations between neuroembryology and behaviour at each stage in developing *Ambystoma*, Coghill (1914) concluded that the peripheral branches of the Rohon-Beard cells are proprioceptive. Each cell sends a peripheral process to both muscle and skin, indicating that they are muscle- and skin-sensory. Centrally an axon projects rostrally from each cell

to connect with internuncial cells. These complete a reflex arc between the Rohon-Beard and the motor cells of the cord. Five increasingly complex behaviour patterns arise sequentially during early development. These patterns of behaviour are elicited by tactile stimulation of the skin and the Rohon-Beard cells form the primary afferent pathway (Coghill, 1914; Riss, 1969). Changes in complexity of the behavioural reflex are described in terms of an ordered sequence in the development of intracentral connections via the internuncials.

These early patterns of behaviour are specific to very young larvae and as the adult pattern of locomotion emerges the role of the primary sensory cells is taken over by the secondary system of dorsal root ganglia. By the end of metamorphosis the Rohon-Beard cells have disappeared (Hughes, 1957).

Much later in ontogeny there is a similar sequential pattern in the development of hind limb movements in *Xenopus laevis* (Hughes and Prestige, 1967) and *Eleutherodactylus martinicensis* (Hughes, 1965). Initially limb movement is tremulous and unco-ordinated. Gradually stepping movements appear and finally the hind legs exhibit the complex movements associated with swimming. These changes have been correlated with the functional development of lumbar ventral horn motor cells (Hughes and Prestige, 1967; Hughes, 1968b). There is evidence that the order in which the motor cells differentiate is related to the segment of the limb which their axons serve. This is deduced from observations of the changing reactions of ventral horn cells to limb removal (see Hughes, 1968a). There are three phases of reaction which can be correlated with stages

in their normal development. In the first phase, amputation has little obvious effect, in the second it immediately raises the normal cell death rate and in the third naturally occurring cell degeneration is halted because cell turnover has ceased within the ventral horn. The changing pattern of cell death shows that:

> "...the cells which innervate each segment of the limb mature in an order which is related to the levels at which their axons terminate in junctions with the muscles."
> (Hughes, 1968a).

In hemimetabolous insects, where development is continuous, the sequential appearance of patterned activity, for example in the flight and song motor patterns of the cricket (Bentley and Hoy, 1970), parallels that in the lower vertebrates. Components of the adult flight motor pattern appear sequentially during development and the final programme is present in the penultimate instar although the wings are still immobile. However, in the development of the nervous system the hemimetabolous insects differ from the vertebrates in one major respect. Although some new cells may be added to the higher centres, the architecture of most of the central nervous system is laid down in embryonic life and the neuron population remains constant throughout development (Edwards, 1969). Thus it appears that the emergence of new behaviour patterns in the Hemimetabola is dependent upon the establishment of functional connections between existing neurons rather than the growth of new cells.

(ii) Neuronal development and the specificity of connections

Convincing evidence for changing sensory connections comes from studies on the development of binocular vision in the frog. In amphibians the retinotectal connection is completely crossed and each eye sends fibres only to the contralateral tectum (see Gaze, 1970). However, in adult frogs a projection to the ipsilateral tectum in an intertectal commissure has been discovered. The contralateral projection is formed early in tadpole development (Sperry, 1963; Jacobson, 1968) but from recent work it is certain that the ipsilateral projection is absent before metamorphosis (Gaze, Keating, Székely and Beazley, 1970; Jacobson, 1971).

During larval life the eyes look out laterally and there is little or no binocular visual field. As metamorphosis approaches the eyes start to move relative to the head until they have adopted their final adult position looking outwards and upwards (Gaze, 1970). There is now considerable overlap of visual fields and Gaze, Keating, Székely and Beazley (1970) have shown that the ipsilateral projection from one eye corresponds with the contralateral projection from the other eye. Therefore, fibres from a particular point in one tectum must grow accurately to the corresponding position in the other tectum. Interaction between the two tecta is necessary for binocular vision and establishment of the ipsilateral pathway occurs at a stage in frog development when binocular vision becomes necessary for range estimation in prey capture.

There are also numerous experimental studies on the development of amphibian skin innervation (Miner, 1956; Jacobson and Baker, 1968, 1969; Baker and Jacobson, 1970). While the authors were primarily concerned with problems relating to specificity and modulation of central connections, the results do have a bearing upon the problem of changing sensory input during ontogeny.

In all vertebrates, cutaneous localization depends upon a topographical projection of the sensory nerves onto the neurons of the central nervous system. If a piece of frog skin is transplanted to another region of the body before metamorphosis and reinnervated by the local nerves, then in the adult, tactile stimulation of that skin results in a misdirected scratch reflex (Jacobson and Baker, 1968; 1969). The implication of the findings is that peripheral connections respecify central connections. However, the misdirected response does not appear immediately after metamorphosis and initially stimulation of the transplanted skin results in a leg movement directed to the point of stimulation. It is concluded that the regenerated peripheral connections do not respecify the central ones in the tadpole. Later, the misdirected response is mediated via the nerves which grow out to innervate the increasing area of skin in the adult. Because skin reinnervation does not modulate the central connections before metamorphosis, Gaze (1970) postulates that 'local sign' only becomes of major importance to the animal when it changes its mode of life at metamorphosis and requires accurate stimulus localization. The same interpretation could be placed upon the results of studies

on the development of the amphibian blink reflex (Weiss, 1942; Kollros, 1943; Székely, 1959). The feature common to the development of the ipsilateral projection, the cutaneous innervation and the blink reflex is that new central nerve connections develop at metamorphosis.

(iii) The developmental mechanics of late neural ontogeny

The normal development of the adult lower vertebrate nervous system prior to and during metamorphosis is dependant upon cell proliferation and selective cell death. The formation of new cells has been known for a long time but the role of cell death in neural ontogeny has only become apparent in recent times (Glücksmann, 1951). These processes have extensively studied in the ventral horn of the spinal cord (see Hughes, 1968a) and in the development of dorsal root ganglia (Prestige, 1965, 1967). Cell counts in the ventral horn of *Xenopus*, at various stages of development, show that whereas there are five or six thousand cells initially, sixty days later the total is reduced to twelve hundred. The main decline in cell numbers takes place in a short period of time corresponding to the time when limb movements develop. The other cells are lost by the process of degeneration. Because there is a cell turnover during these sixty days the total number of cells lost may be as great as ten thousand (Hughes, 1968a). Thus for every cell which reaches maturity, eight or nine others have been lost. A turnover ratio has also been calculated for the lumbar dorsal root ganglia (Prestige, 1965) and here one in three cells persists in the adult. It seems

that there are many transient nerve-muscle connections during development that do not conform to the final pattern of relationships. Stimulation of developing motor nerves in *Xenopus* tadpoles at first evokes a wide range of movements that gradually becomes narrowed to the final pattern of response. This change in behaviour can be explained in terms of central and peripheral cell selection (Hughes, 1968b) but the necessity for, and meaning of, such a great cell turnover is difficult to understand.

The holometabolous insects also show very great changes in the nervous system during metamorphosis but they are unlike those in the vertebrates. Numerous studies (e.g., Heywood, 1965; Nordlander and Edwards, 1968, 1969) have shown extensive production of new neurons from neuroblasts which persist until metamorphosis and although some larval nerve cells persist in the imago (Panov, 1963) much of the larval system degenerates (Edwards, 1969). In addition, the imaginal discs contribute entirely new components to the nervous system. For these reasons it is impossible to study a sensory system before and after metamorphosis because larvae and adults have virtually independant nervous systems and repertoires of behaviour.

(iv) Retinal ganglion cell anatomy and physiology, an example of the changing sensitivity of a receptor during development

A recent study of frog retina before and after metamorphosis (Pomeranz and Chung, 1970) provides convincing evidence that structural modifications of ganglion cell anatomy change the sensitivity of the eye to certain types of stimuli. Four types

of visual form detector units can be recorded from frog optic nerve (Lettvin, Maturana, Pitts and McCulloch, 1961). Classes 1, 2 and 3 respond best to particular different moving forms while class 4 responds to decreasing light intensity. These four types of response are thought to represent activity in each of four classes of ganglion cell distinguishable on the basis of dendritic tree anatomy. Responses from tadpoles (Pomeranz and Chung, 1970) were similar to those reported in the adult for classes 2, 3 and 4. The major differences between the tadpole and the adult were the total absence of class 1 fibres (edge detector units) and the absence of class 2 fibres (convex edge detector units) in the periphery of the larval retina. These findings were correlated with the absence of one type of ganglion cell and with the restricted distribution of another. It is not certain how these changes affect the integration of visual signals in the tadpole but the study provides convincing evidence that the structure and physiology of frog retina is modified at metamorphosis.

II. Studies on the lateral line systems of fishes and amphibians

The literature on the lateral line system is extensive and several good reviews are available (Dijkgraaf, 1934, 1963; Wright, 1951; Lowenstein, 1957; Cahn, 1967). Although the early development has been described, little is known of lateral line function in larval anurans. Information relevant to the function of the system in adult *Xenopus* is summarized here as

a basis for the investigation of lateral line anatomy, physiology and function in *Xenopus* tadpoles.

(i) Lateral line function in fishes and amphibians

The existence of the lateral line system has been known since the middle of the seventeenth century (Stenonis, 1664; quoted from Johnson, 1917) when it was thought to be a mucous secreting organ. Convincing evidence for its role in sensory physiology was obtained only recently although a sensory function had been predicted from histological evidence much earlier (Leydig, 1850). The first recordings from lateral line nerve were made by Hoagland (1933a - d, 1934) in catfish where changes in temperature were shown to modify the frequency of the 'spontaneous' discharge. Units responding phasically to pressure changes, surface ripples, irregular water currents and movements of the fishes own trunk were incorrectly assumed to represent a second class of lateral line receptors. The classic work of Sand (1937) demonstrated in the ray that the spontaneous discharge was modified by a slow movement of fluid in the lateral line canal. Flow in one direction accelerated the firing rate while flow in the opposite direction inhibited it. Receptors with antagonistic directional sensitivities are mixed within lateral line organs. Subsequent studies on the free standing lateral line organs of amphibians (Dijkgraaf, 1956; Görner, 1961, 1963; Flock, 1971) and teleost canal organs (Flock, 1965) have confirmed these findings but there has been considerable debate over the effective stimulus for the lateral line system (see Lowenstein, 1967). There is evidence

that the lateral line system responds to low frequency vibrations (see e.g. Sand, 1937; Suckling and Suckling, 1950) and, as the lateral line system is embryologically and phylogenetically linked with development of the inner ear, this suggested that lateral line organs are sound receptors. Alternatively, the organs may detect water displacement (see e.g., Dijkgraaf, 1934, 1963). Most sound sources, however, produce both water displacements and pressure changes. The elegant theoretical and experimental arguments of Harris and Bergeijk (1962) have now convincingly proved that the lateral line system is not sensitive to pressure changes caused by an oscillating sound source and that the effective stimulus is water displacement.

(ii) The lateral line system in adult *Xenopus*

Structure and function of the lateral line system has been extensively studied in adult *Xenopus*. Organs are grouped in plaques (Escher, 1925; Murray, 1955) within which they are separated from each other by tactile organs (Calabresi, 1924) (Fig. 39) which channel water currents onto the lateral line organs (Görner, 1961) and may have a protective function. Rows of plaques make up a lateral line and a common nerve trunk supplies the row with branches to each plaque.

Every organ contains receptor cells (hair cells), each with a hair process projecting from its free surface. The hair processes of the cells in one organ are enclosed in a gelatinous cupula (Dijkgraaf, 1963). Within each hair process there is a single kinocilium with the usual 9 + 2 arrangement of ciliary filaments, and a group of stereocilia to one side of

it (Wersäll, 1956). The stereocilia are about two-thirds of the diameter of the kinocilium and contain an irregular arrangement of longitudinal filaments.

Electrophysiologically it has been shown that there are two classes of receptor cell, sensitive to water displacements in opposite directions (Görner, 1963). This correlates with the anatomical arrangement of the receptor cell hair processes. Half of the hair cells have the kinocilium on one side of the stereocilia and half have the kinocilium on the opposite side (Dijkgraaf, 1963; Görner, 1963). In one region of the labyrinth where all hair cells have the same morphological polarization, deflection of the hair process towards the kinocilium causes depolarization of the cell and the reverse displacement causes hyperpolarization (Lowenstein and Wersäll, 1958). The maximum sensitivity of each lateral line plaque is approximately at right angles to its long axis although there is some variation because all organs within one plaque do not always have exactly the same orientation of their hair processes (Görner, 1961, 1963).

The nerve fibre running from each plaque to the main nerve trunk always contains two large (thick) myelinated fibres (8 - 10 μm in diameter); a bundle of non-myelinated fibres; and often but not always one or more small (thin) myelinated fibres (0.5 - 1.0 μm in diameter) (Murray, 1955; Flock, 1967). The two large fibres are sensory (Görner, 1967) and there are non-granulated synaptic terminals contacting the lower regions of the hair cells (Delaveuve and Szabo, 1966; Flock, 1967). These endings are so-called because of the absence of granulated

vesicles in their cytoplasm. Similar endings have been described in teleost lateral line organs (Trujillo-Cenóz, 1961; Hama, 1965; Flock, 1965) where they are thought to be afferent terminals. In *Xenopus*, although the exact pattern of innervation is unknown it is thought that non-granulated terminals are the endings of the large myelinated fibres. The small myelinated fibres are efferent (Görner, 1967) and stimulation of efferent units causes inhibition of spontaneous afferent activity (Russell, 1968). Inhibitory type granulated endings (Engström, 1958) contact the hair cells in *Xenopus* and teleost organs, but it is not known if they are the terminals of the small myelinated fibres. They are almost certainly the terminals of efferent fibres because similar endings in the sensory epithelium of the ear degenerate after the section of efferent nerves (Engström and Fernández, 1961; Iurato, 1962; Bairati and Iurato, 1962; Kimura and Wersäll, 1962; Spoendlin and Gacek, 1963; Smith and Rasmussen, 1963; Hillman, 1969a; and others). The function and destination of the non-myelinated fibres is uncertain although an inhibitory function has also been attributed to them (Harris and Flock, 1967).

Nothing is known of the structure and function of the sense organs in *Xenopus* larvae, but the fine structure of organs in the leopard frog tadpole agrees well with the above description (Jande, 1966). The larval lateral line nerves are undescribed.

(iii) The lateral line system in behaviour

Although an increasingly sophisticated picture of lateral line physiology has been built up (see e.g. Flock, 1971) analyses of function using behavioural tests are still relatively crude and the early observations are still important. In *Ambystoma punctatum*, a small jet of water directed at the lateral line organs of the head elicits a snapping response, the animal turning towards the jet (Scharrer, 1932; Detwiler and Copenhaver, 1940). It was suggested that the animal is heavily dependant upon the lateral line system for prey capture and that the eyes are of little importance in feeding behaviour.

The lateral line system can detect an object in the environment which moves relative to the animal and so functions as an organ of *Ferntastsinn* or *touch-at-a-distance* (Dijkgraaf, 1934). Similarly both fish and amphibia respond to local disturbances at the water surface (Schwartz, 1965, 1967, 1970; Dijkgraaf, 1967) and they turn towards the source of the surface waves. In surface-feeding fishes the responses can be elicited only in fish which are very close to the surface (Schwartz, 1970). Both touch-at-a-distance and surface wave stimuli can be classified as near-field effects. The role of the lateral line system as a temperature receptor has been discredited although blinded minnows can discriminate between warm and cold jets of water (Dijkgraaf, 1940, 1943). The response is abolished if the spinal cord is transected showing that temperature perception depends upon general cutaneous endings of spinal nerves (Dijkgraaf, 1963). Nothing is known

of the role of the lateral line in anuran tadpoles and it is difficult to predict a function because they are mostly vegetarians.

(iv) The early development of the lateral line system in amphibia and the distribution of rows in *Xenopus* larvae

Harrison (1903), by transplanting the tail of a black tadpole onto the trunk of a light brown species, established that the lateral line sense organs develop from ectodermal rudiments located on the head. These migrate along the prospective lines of the system and the sense organs are formed from the migrating cells. Harrison's original observations were confirmed and expanded by Stone (1922, 1923, 1924, 1925, 1928a, b and c, 1929, 1931, 1933, 1935, 1937) working with *Ambystoma punctatum*. He showed that the various lines of sense organs each originate from a separate part of one of two placodes on the head which migrate to form definitive lines. These placodes first appear in relation to the primordium of the inner ear. The preauditory placode gives rise to the anterior rows, and the postauditory placode to the posterior rows. As the tissue migrates, groups of cells are deposited which give rise to both the sensory and supporting cells of individual organs. The overlying ectodermal cells are pushed aside and the sensory cells become exposed at the surface. These primary organs give rise to other organs by a budding process to form plaques which in the adult share a common innervation (Murray, 1955). Nieuwkoop and Faber (1967) show that the lateral line system develops in a similar way in *Xenopus*.

No physiological studies have been made in any anuran tadpole prior to metamorphosis, so it is not known when the lateral line nerves establish functional connections with the receptor cells or at which stage in development the lateral line system becomes functional.

The lateral line system was first described in *Xenopus* larvae at the beginning of the century (Bles, 1902, 1904) but because of the translucent qualities of the tadpole only a few of the many organs were noticed. Similarly even Escher (1925), who carried out a vast survey of lateral line systems, failed to describe the larval organs. Changes at metamorphosis were first observed by Paterson (1939) who noticed that certain of the head organs became regrouped around the eyes. Nieuwkoop and Faber (1967) and Shelton (1970) have completed the description of the plaque rows. They extend dorsally, laterally and ventrally over the surface of the head, trunk and tail. The various rows have been named using the system adopted by Escher (1925) for other amphibians. Large parts of the system, especially the lines on the tail and parts of the head are lost at metamorphosis. The remaining organs are incorporated into the adult system but some rows are reduced or reorganized (Figs 3 and 35).

III. Aims of the present investigation

At metamorphosis the lateral line organs of the tadpole are redistributed not only in the supra-orbital row (Paterson, 1939) but also on all parts of the body (Shelton, 1970). In order to interpret the effects of these positional changes on

sensory input, it is necessary to know whether the organs of the tadpole are directionally sensitive. This study is therefore largely concerned with the response of the system to water displacements although the lateral line has been implicated in the detection of other parameters such as temperature (Murray, 1956) and ion concentration (Onada, Hashimoto and Katsuki, 1970).

The development of lateral line nerves and the fine structure of tadpole organs is described and the function of the afferent and efferent fibres is investigated electrophysiologically. Changes occurring in the lateral line system at metamorphosis are detailed and behavioural responses to directional near-field stimuli are examined before and after metamorphosis.

Finally the innervation of the adult hair cell is investigated using medium high voltage electron microscopy and reconstruction techniques to show that non-granulated endings are afferent synapses and granulated endings are associated with the small myelinated efferent fibres.

CHAPTER II

ANIMAL CULTURE AND GENERAL METHODS

This chapter describes techniques of general application, more specific methods are covered in the relevant sections of the thesis. Procedures for the preparation of fixatives, salines, stains, etc., are given in Appendix I found at the end of the thesis.

I. Animal culture

Live adult toads were obtained from L. Haig and Co. Ltd., Newdigate, Surrey, United Kingdom, and The Division of Inland Fisheries, Jondershoek, South Africa. The first supplier delivered the animals packed in damp moss, and the latter provided a fish transporting tank in which 50 toads were dispatched in 5 gallons of water. Both methods of transport are good and mortality was low. The animals were isolated on arrival and daily inspections were made for disease. In the laboratory they were kept in wire netting covered 20 gallon fibreglass tanks of tapwater. The water was allowed to stand for two days to allow chlorine to evaporate before introducing the toads. The temperature of the animal room was kept at 20°C. Feeding was carried out once a week, the food being chopped liver and worms when available. The tanks were cleaned on the following day.

Breeding was induced using chorionic gonadotrophin injected into the dorsal lymph sac prior to mating. Males are given doses of 100 I.U. of hormone on each of three consecutive days and the female is given one dose of 500 I.U. on the third day. The animals are placed together and mating usually takes place within eight hours. Hormone can be obtained from a number of sources, ours came from Sigma Chemical Company U.S.A. and was supplied in phials containing 2,500 I.U. of solid hormone. By dissolving this in ten ml of distilled water each dose is easily measured and only small volumes of fluid have to be injected. Mating normally takes place at night and the adults should be removed the following morning. The eggs should be aerated and they hatch within two or three days. After about four to six days the larvae begin to swim actively around the tank and feeding should commence at this stage. The most convenient food is a suspension of finely ground nettle powder (*Urtica* sp.) which is added to the tank every second day. Enough food should be added to make the water opaque. Tanks can be cleaned once a week if the food requirement is accurately judged. Powdered ox liver or hen's egg is equally suitable as a food but more regular cleaning of the tank is required. The nettle powder can be obtained from herbalist stores, our supply was obtained from R. Brookes and Co., 27 Maiden Lane, Covent Garden, London, W.C.2, U.K. A useful guide for the care and maintenance of *Xenopus* in the laboratory has been prepared by Gurdon (1967) and is a valuable source of information. Tadpoles were staged according to the Normal Table of Nieuwkoop and Faber (1967).

II. Anaesthesia

For histological examination of tissues, animals were anaesthetized prior to fixation. For tadpoles a one in 10,000 solution of tricaine methanesulphonate (MS 222) in distilled water was used. It is obtainable from Sandoz Pharmaceuticals, Hanover, New Jersey, U.S.A. For adults a stronger solution is recommended and about one teaspoonful in half a bucket of water brings about anaesthesia in five to ten minutes. Trichlortertiarybutyl alcohol (chloretone) can also be used (Cole, 1922); a one part in 1,000 of distilled water anaesthetizes a tadpole in ten minutes.

MS 222 was also used to anaesthetize animals prior to dissection for physiological recording. Most specimens were subsequently decerebrated and immobilized with d-tubocurarine injected into the lymph spaces beneath the skin. 0.1 ml of a 0.3 per cent solution in distilled water was used for newly metamorphosed animals and the dose was increased to 1.0 ml of solution for large animals. Tadpoles were curarized with a correspondingly smaller dose (0.01 ml of 0.3 per cent solution) injected into the tail musculature, or by immersion in a bath of 0.03 per cent d-tubocurarine. After decerebration and immobilization the animal was allowed to recover from the anaesthetic.

Curarization can interfere with synaptic transmission, and for this reason recordings were also obtained from uncurarized animals under light MS 222 anaesthesia. However, anaesthetics reduce the level of 'spontaneous' activity in the

lateral line nerve (Sand, 1937), a result which was confirmed in this study. The normal level of activity returns as the effects of the anaesthetic wear off but even decerebrate animals struggle without anaesthetic. This situation obviously leads to recording difficulties but adequate records were obtained and they were similar to those from curarized preparations.

III. Dissection and nerve display

The lateral line nerves are easily accessible lying just beneath the skin and closely applied to it. For recording from lateral line nerves, the overlying skin is carefully deflected and the nerve is separated from it using fine glass hooks. Throughout the operation and during subsequent recording, the animal is covered with physiological saline prepared after the formula of Russell (1968) (see Appendix I).

IV. Anatomical techniques

Many of the anatomical methods employed are dealt with in the chapters concerning electron microscopy, the relevant formulae and staining procedures appearing in Appendix I.

Figure 2 was prepared from wax embedded material fixed with Carnoy fixative and dehydrated in the usual way (Pantin, 1964); it is a section from one of many brains stained by the Holmes method, the schedule for which appears in Appendix I.

CHAPTER III

THE MORPHOLOGY AND ULTRASTRUCTURE OF THE LATERAL LINE SYSTEM IN LARVAL *XENOPUS*

The following account describes the morphology and fine structure of the lateral line system in *Xenopus* tadpoles. Most of the published work relating to *Xenopus* lateral line refers to the adult system. Thus, various aspects of the gross morphology of the post-metamorphic system have been described by Escher (1925), Horst (1934), Paterson (1939) and Murray (1955). The ultrastructural studies of Dijkgraaf (1963), Delaveuve and Szabo (1966), Flock (1965 and 1967) are also restricted to lateral line in the adult. Undoubtedly the lack of descriptions of the system in larval stages is attributable to the transparency of the skin which makes the organs difficult to see. The most accurate accounts of the locations of lateral line rows in the larva are those of Nieuwkoop and Faber (1967) and Shelton (1970).

There is no comprehensive study of the fine structure of lateral line nerves or organs at larval stages although a few details are available (Shelton, 1970). There is a description of organ ultrastructure in *Rana pipiens* tadpoles (Jande, 1966) but this account is not necessarily accurate for other Anurans. Because the present study is concerned with possible changes in the lateral line at metamorphosis an accurate description of the system in *Xenopus* larvae is essential.

MATERIALS AND METHODS

I. Whole mount skin preparations

Animals were skinned at various stages of development in order to map the distribution of lateral line organs. Osmium tetroxide fixative was pipetted onto the skin and material being prepared for whole mounts was allowed to darken noticeably before being removed and dehydrated in acetone. Skins were laid out on microscope slides and covered with small pieces of glass to keep them flat during dehydration. They were finally mounted in Araldite under cover slips on microscope slides. The osmophilic properties of the organs and nerves made them stand out against the paler background.

II. Techniques for electron microscopy

Tissue prepared for electron microscopy was fixed with glutaraldehyde followed by osmium tetroxide solutions, or with just osmium tetroxide alone. Prefixation with buffered glutaraldehyde provided the best preservation of cytoplasmic detail but satisfactory preservation of myelin sheath was not consistently achieved when glutaraldehyde was used in the fixation procedure. It is felt that this may be due to problems peculiar to the lateral line nerve as elsewhere (see for example: Hillman, 1969b; Sotelo, 1969) myelin has been satisfactorily preserved in Anura using glutaraldehyde fixatives.

Animals were anaesthetised and cold fixative was allowed to flow over the relevant tissue. The tissue was carefully removed

and the fixation procedure continued in glass vessels at 4.0°C. Details of the fixatives appear in Appendix I. Fixed tissue was dehydrated in an acetone series using 20, 50, 70, 90 and 100 per cent solutions. Tissue was left for 20 minutes at each of the first four stages, the acetone being changed three times during each of the 20 minute periods. It was placed for one hour in absolute acetone to complete dehydration and was then transferred to a mixture of equal parts acetone and freshly prepared Araldite mixture. The mixture was left for the acetone to evaporate over night. The material was then transferred to fresh Araldite in a plastic container and baked for 24 hours at 60°C. After trimming, the block was ready to section. Instructions for preparing Araldite of the appropriate hardness are given in Appendix I. Following the above procedure the block was roughly shaped with a dental drill and trimmed in the standard fashion with a razor blade. Difficulties in sectioning after embedding in this fashion are not normally due to incorrect consistency of the Araldite but to poor quality glass knives. Blocks were sectioned on a Sorvall Porter-Blum MT 1 ultramicrotome for light microscopical examination and on a Reichert OMU 2 ultramicrotome when ultrathin sections were required. Thick sections prepared for light microscopy were mounted on slides and stained with toluidine blue after the method of Trump, Smuckler and Benditt (1961) (see Appendix I).

Several electron dense stains were used for fine structural work. Some tissue was block-stained with a saturated solution of uranyl acetate in 70 per cent acetone, but generally sections were surface stained on mesh grids using uranyl acetate and lead

citrate. A saturated uranyl acetate solution in distilled water was used. Lead citrate solution can be made directly by dissolving solid lead citrate in distilled water or indirectly according to the method of Reynolds (1963). In both cases the stain is made alkaline by adding sodium hydroxide. Procedures for preparing lead citrate are given in Appendix I. Drops of uranyl acetate and lead citrate were placed separately on a sheet of dental wax and the sections were stained by floating the grids section-side down on the drops for periods of four minutes on each of the solutions. They were placed on the uranyl acetate first and briefly washed before transfer to the lead solution. After removing from the lead citrate the grids are washed in 0.02 N sodium hydroxide followed by a rinse in distilled water. The whole staining procedure was carried out in a closed glass vessel. Prepared sections were allowed to dry after staining and they were examined in a Hitachi HU 11E electron microscope.

RESULTS

I. The central connections and the general morphology of the lateral line system in the tadpole

The lateral line system of the tadpole is naturally divisable into anterior and posterior rows or lines. The two halves of the system receive their innervation from two separate cranial nerves which both enter the medulla oblongata as single roots. Because of the fusion of cranial nerves and their ganglia in the Amphibia it is difficult to number the lateral line roots (Horst, 1934)

and for this reason it is simpler to refer to them as the anterior and posterior lateral line roots. The anterior lateral line nerve enters the brain between cranial nerve five and nerves seven and eight. The posterior lateral line nerve enters the medulla in a position antero-dorsal to the cranial nerve roots nine and ten (Fig. 2A). On entering the medulla the lateral line nerves split up into a number of fascicles (Fig. 2B) and the individual nerve fibres divide into two forming ascending and descending branches (Fig. 2C). These branches run in a tract ventral to the crista cerebellaris.

Peripherally both the anterior and posterior lateral line nerves each pass through separate cranial ganglion complexes before branching to innervate individual lateral line rows. The anterior lateral line nerve supplies three rows, the supra-orbital, the infra-orbital and the hyomandibular, each of which has further subdivisions. The posterior lateral line nerve divides peripherally to supply five rows, the occipital, the aortic, the lower lateral, the middle lateral and the upper lateral lines. The lower lateral line has several subdivisions. In addition to the rows there are anterior and posterior auditory groups. The positions of these rows have been plotted from whole mount skin preparations (Fig. 3) and from superficial examination of undissected tadpoles. In both cases the location of individual organs was made clear by staining the skin with osmium tetroxide.

Lateral line organs are arranged in plaques (Fig. 4). These are groups of organs all innervated by the same nerve fibres. The number of organs in each plaque increases during development,

thus at stage 49 there may be only one organ per plaque while at the time of metamorphosis there can be as many as ten organs in a plaque. Adjacent organs in the larval plaques are separated by epithelial cells. The increase of number of organs is apparently due to a budding process in which existing organs split to give rise to additional ones. The number of plaques remains steady until metamorphosis. There is considerable variation in the exact positions and number of plaques in a given row in different animals and on left and right sides of the same animal.

II. The fine structure of lateral line organs in the tadpole

(i) General morphology

The following account refers to lateral line organ fine structure at developmental stage 54. At this stage the larval organization is already well established and metamorphosis is still a long way off.

The position of each organ is indicated by a slight bump on the skin surface (Fig. 5A). Each organ is composed of a group of about two dozen receptor cells (hair cells) and various supporting cells. Hair-like processes emanate from the apical parts of the receptor cells (Fig. 5B). The supporting cells can be classified after the manner of Chezar (1930) into three classes: mantle, basal and sustentacular cells. The mantle cells surround the organ and separate the receptor cells from the epidermal cells of the skin. The basal cells are situated underneath the receptor cells and separate them from the basement lamella, a collagenous

layer situated immediately below the skin. The sustentacular cells are much less obvious and separate individual receptor cells from one another. These details of general morphology are illustrated in Figure 6.

(ii) Receptor cell structure

Receptor cells are pear-shaped being about 10 - 15 μm long and tapering from about 4 - 7 μm wide at the base to 3 μm or less at the apex (Fig. 7A). A large spherical nucleus is situated in the basal third of the cell. Three types of structure project from the apical surface of each receptor cell; these are: a single kinocilium, a group of stereocilia, and a few microvilli (Figs 5C and 7A). The cilia from each cell form what is known as a hair cell bundle or hair process. The kinocilium is about 0.25 μm in diameter and has the normal nine plus two arrangement of microtubules. Its basal body is placed to one side of the rootlets of the stereocilia and the basal foot points away from the stereocilia (Figs 7B and C). The number of stereocilia varies from cell to cell but is between 20 and 70. Stereocilia are of variable lengths, those nearest to the kinocilium being up to 1.75 μm long while those on the far side of the receptor cell can be as short as 0.4 μm (Fig. 7B). All have a diameter of about 0.2 μm. They do not have the nine plus two arrangement of tubules seen in kinocilia but fibrils are visible running the lengths of their structure. The stereocilia are bounded by a membrane continuous with that of the rest of the cell and they contain fairly densely staining cytoplasm. They have parallel sides for most of their length but taper towards the base where the fibrils

come together to form a dense rootlet (Fig. 8). The rootlets of the stereocilia are short and enter the cuticular plate, a specialised densely staining region at the apex of each receptor cell (Fig. 7A). This region has an amorphous structure but microtubules can be seen running from its lower margin into the upper regions of the receptor cell (Fig. 9). Cut in horizontal section the cuticular plate has a horse-shoe-shaped appearance and a regular arrangement of sterocilium rootlets each surrounded by less densely staining cytoplasm (Fig. 10). The basal body of the kinocilium is always situated on the concave side of the cuticular plate. The cupula, if present in tadpoles, is not easily visible in electron micrographs but vesicles and electron dense material are often present between the stereocilia (Figs 8 and 10). Just below the cuticular plate region the receptor cells are attached to adjacent supporting cells by a network of desmosomes (Fig. 13D). Lower down the cells are less tightly bound and often there are quite large intercellular spaces. The cytoplasm between the nucleus and the cuticular plate is characterized by the presence of elaborate Golgi complexes, multivesiculate bodies, elongate mitochondria, large cysternae of the endoplasmic reticulum containing an amorphous material, and a large amount of rough endoplasmic reticulum. Some of these features are illustrated in Figures 7A and D and Figure 9. Below the nucleus the cytoplasm is packed with synaptic-type vesicles approximately 350 Å in diameter. The mitochondria in this region are large, spherical and have a complicated arrangement of cristae. These mitochondria do not occur elsewhere in the cell and they may have some specialised function. In this

regard it is possible that they are concerned with synaptic function as they are situated close to hair cell contacts with nerve terminals (Figs 7E and F).

(iii) Dark receptor cells

Some receptor cells in each lateral line organ stain extremely densely with toluidine blue and have an appearance suggestive of degenerative change. Electron micrographs reveal a high density of cytoplasmic contents, complex membrane whorls and large mitochondria (Figs 11 and 12A - D). Stereocilia are lacking and the kinocilium stains as darkly as the main part of the receptor cell. The basal regions of the cells are associated with nerve terminals in the normal way (see below).

(iv) Morphological polarization of the receptor cells in one organ

Individual receptor cells are morphologically polarized by the asymmetrical arrangement of their hair cell bundles (Fig. 5C), by the position of the cuticular plate in the top of each cell (Fig. 10) and by the location of the basal foot of the kinocilium (Fig. 7C). Lowenstein and Wersäll (1959) showed that there is a similar polarization of the hair processes in the sensory epithelia of the elasmobranch labyrinth. Furthermore they showed an important correlation between this morphological asymmetry and the physiological finding that hair cells have a strongly directional sensitivity to displacement of the sensory hairs. If there is a directional sensitivity of the lateral line organs in *Xenopus* tadpoles one might expect to see this reflected in the overall arrangement of the hair cell bundles in one organ. For this

reason, the tops of all the receptor cells of a single organ were reconstructed from serial sections (Figs 13A - D). The completed reconstruction shows that the hair cell bundles are arranged in a non-random fashion. Approximately half of the cells have their kinocilium on one side of the stereocilia and the other half have the kinocilium on the opposite side (Fig. 14). However, the distribution of the two classes of cell within an organ does seem to be random and is in contrast to the situation in *Rana pipiens* tadpoles (Jande, 1966). Here, the two cell types are in separate groups; all of one type are on one side of the organ's midline and all of the other type are on the other side. In *R. pipiens* each kinocilium is on the side of its receptor cell nearer to the midline. This is not the case in *Xenopus* where cells of each type occur on both sides of the midline.

(v) Supporting cells

The classification of supporting cells into mantle, basal and sustentacular cells is essentially a geographical one and the fine structural detail of all is similar. Up to three layers of mantle cells surround the organ (Fig. 6). They enclose the organ rather like the outer layers of an onion. The inner mantle cells are attached to the basement lamella at their bases but the outer layers of cells do not reach the basement lamella and are normally attached to epidermal cells. Their cytoplasm contains Golgi complexes, elongate mitochondria, rough and smooth endoplasmic reticulum with enlarged cysternae and vesicles of various sizes. The peripheral ends of the mantle cells are often highly vacuolated. The basal cells are attached to the

basement lamella forming a single layer of cuboid cells beneath the receptor cells. Cytoplasmic details are similar to those of the mantle cells (Fig. 15A and B). The sustentacular cells are small flattened cells lying between adjacent receptor cells. They separate the receptor cells from one another (Fig. 7A).

III. Lateral line organ innervation in the tadpole

(i) The nerve trunks

The lateral line nerve trunks in tadpoles from stage 49 onwards contain two morphologically distinct classes of axon. These are myelinated and unmyelinated fibres (Fig. 16A). There is a continuous spectrum of myelinated fibre diameters ranging from 1.5 - 10 μm in diameter in the youngest animals and increasing to 3 - 15 μm in late stage tadpoles. From stage 54 to stage 60 all fibres are either clearly unmyelinated or fully myelinated and no intermediate stages of development are seen. This suggests that the fibre population of the lateral line nerves is stable for a large part of the larval life.

The medullated fibres have a typical myelin sheath which shows the characteristic periodicity of structure (Davison and Peters, 1970). The cytoplasm of the Schwann cells contains the large quantities of rough endoplasmic reticulum and mitochondria usual in metabolically active and developing cells (Fig. 16B). The unmyelinated fibres occur in bundles of six to ten axons closely enveloped in glia. Microtubules and microfilaments are distinguishable within the axons (Fig. 16C). Filaments, probably collagen, are present between the axons in the nerve trunks and they presumably have a supporting role.

Each lateral line nerve trunk is enclosed in investing membranes and fibres only exit from it in small bundles of several axons. These bundles innervate single lateral line plaques. For this reason it seems that all the axons in lateral line nerve trunks are concerned with lateral line function. The nerve bundle innervating a single tadpole plaque contains a pair of myelinated axons with similar diameters and a bundle of three to six unmyelinated axons invested in a single glial element (Fig. 17). These nerve fibres branch to all the organs in the plaque, the myelinated fibres losing their sheaths as they penetrate the basement membrane beneath each organ.

(ii) Nerve endings in the lateral line organ

Nerves innervating each organ pass up through the basement lamella and travel between the supporting cells to the basal part of the receptor cells. At this level all nerves are naked and they are morphologically indistinguishable (Fig. 18). For this reason it is impossible to relate the different types of synaptic structures associated with the hair cells (see below) to the different classes of axons in the lateral line nerve trunks. The nerve terminals form two morphologically distinct types of contact with the hair cells. These will be referred to as Ne 1 and Ne 2 to conform with the precedent set in the literature (see for example: Engström and Wersäll, 1958). No synaptic contacts between the two types of nerve ending were seen, all synaptic structures were located at junctions of nerve endings with hair cells.

(iia) The Ne 1 type endings

This type of ending, known in other lateral line systems, is alternatively referred to as a non-granulated ending indicating an absence of granulated vesicles in the terminal (Hama, 1962). Characteristically Ne 1 endings contain mitochondria and few vesicles of any sort. The endings are closely associated with the bases of the receptor cells and there are specialized regions of contact between the two. At these sites the receptor cell membrane is thickened in a distinctive fashion and is separated from the nerve ending by a synaptic cleft of about 200 Å width. The membrane of the nerve ending is also thickened and there appears to be amorphous material within the synaptic cleft. The other very distinctive feature of this class of contact is the presence of synaptic bars or bodies within the receptor cell and close to the regions of membrane modification. These organelles are spherical with a diameter of 0.3 - 0.4 μm and have a high stain affinity. They seem to be amorphous, have no visibly organized substructure and are encircled by vesicles with an outside diameter of about 350 Å (Fig. 19C and D). One hair cell has many of these synaptic sites upon its surface but from random electron micrographs it is impossible to know whether they are all associated with a single non-granulated ending or whether several such endings contact each hair cell.

Non-granulated endings are also found in the lateral line system of fish (Flock, 1965). Comparing their structure with similar endings in other parts of the acoustico-lateralis and the visual systems Flock concludes that they represent sites of

afferent transmission. This generalization is extended here to include the non-granulated endings of the tadpole.

(iib) The Ne 2 type endings

This class of ending contains a few mitochondria and large numbers of synaptic vesicles approximately 350 Å in diameter. Sometimes larger vesicles containing a granule are present. Consequently in fish this type of terminal has been referred to as a granulated ending (Hama, 1962). The nerve endings contact the receptor cell in only one or two places. At these points the ending and receptor cell are separated by a synaptic cleft having a uniform width of about 200 Å. There is no thickening of the synaptic membranes. Within the receptor cell and closely applied to the receptor cell membrane is an elongated flat sac or cistern. Vesicles are concentrated next to the synaptic membrane in the nerve ending (Figs 7F and 19A and B). A single receptor cell may contact one or more of these granulated endings which may or may not be branches of the same nerve.

Engström (1958) deduced from the resemblance of granulated terminals of the inner ear with the presynaptic terminals within the central nervous system that they were efferent in function. Subsequent experimental work supports this prediction (see for example: Smith and Rasmussen, 1963; Spoendlin and Gacek, 1963). Without evidence to the contrary it is assumed that the granulated endings in the tadpole are also the terminals of efferent nerves.

(iii) Other possible nerve endings

Other classes of ending are known in the sensory epithelia of the labyrinth of the lamprey and the ray (Lowenstein, Osborne and Wersäll, 1964; Lowenstein, Osborne and Thornhill, 1968). On three occasions during the course of the present study structures were observed which might possibly be classified as the so-called electron dense endings (Ne 3) of Lowenstein et al (Fig. 20). In the lateral line system they are small electron dense processes about 0.25 μm in diameter. They are found between receptor cells peripheral to the other types of ending and appear to contain microtubules with an outside diameter of about 250 Å. It must be stressed, however, that there is no evidence to confirm that these structures are of neural origin.

Major details of receptor cell structure and connections with the terminals of known neural origin are summarized in Figure 21.

SUMMARY AND CONCLUSIONS

Lateral line plaques are well developed in the stage 54 tadpole and appear anatomically fully differentiated. The morphological polarization of receptor cells provides anatomical evidence for directional sensitivity. Darkly staining receptor cells are found in each organ. Similar cells are found in taste buds after denervation (Olivieri-Sangiâcomo, 1970). The present finding may be evidence for a turnover of receptor cells in the lateral line organ.

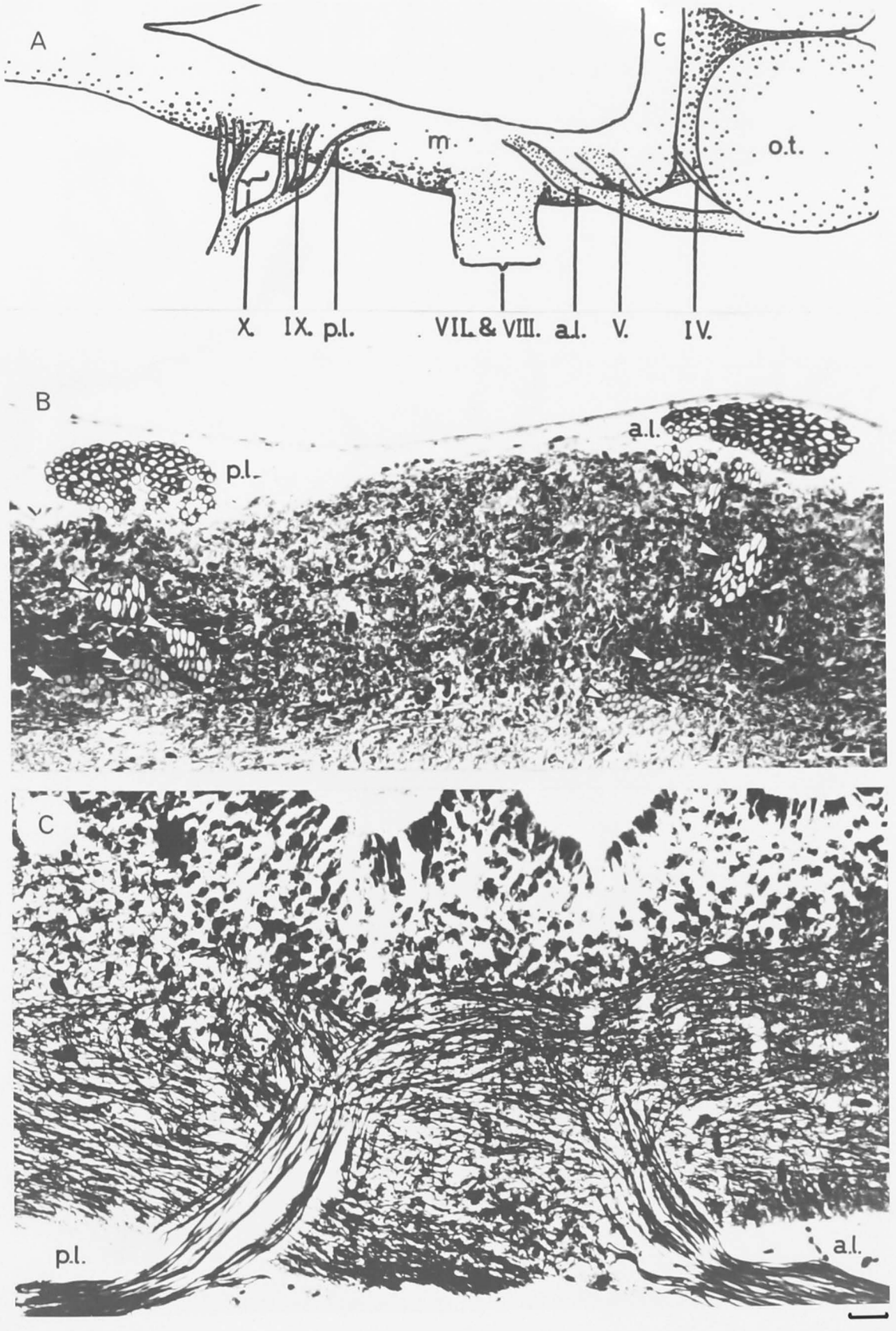
There are both myelinated and unmyelinated fibres in the lateral line nerves of the tadpole. It is likely that the paired myelinated fibres innervating each plaque correspond to the thick fibres in the adult (Görner, 1963) and are therefore sensory. Two types of nerve terminal on the hair cells are thought to have afferent and efferent functions. A third type of ending is described which may correspond to the Ne 3 type of the labyrinthine epithelium.

Figure 2

(A) Dorsal view of the right sides of the medulla oblongata (m), cerebellum (c) and optic tectum (o.t.) of a stage 58 *Xenopus* brain. Various cranial nerve roots have been numbered and the anterior and posterior lateral line nerve roots (a.l.; p.l.) are indicated. (Modified after Horst, 1934.)

(B) Vertical longitudinal section of a stage 56 tadpole brain to show separate fascicles of the lateral line roots (arrowed) entering the medulla oblongata. (1 µm Araldite section stained with toluidine blue). a.l. = anterior lateral line nerve; p.l. = posterior lateral line nerve.
Scale 50 µm

(C) Horizontal longitudinal section of a stage 54 tadpole brain to show the right lateral line nerves entering the medulla. Each sensory nerve divides to form ascending and descending branches which run in a longitudinal tract beneath the crista cerebellaris. (10 µm wax embedded section stained by the Holmes silver method.)
Scale 50 µm


A
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m
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IX.
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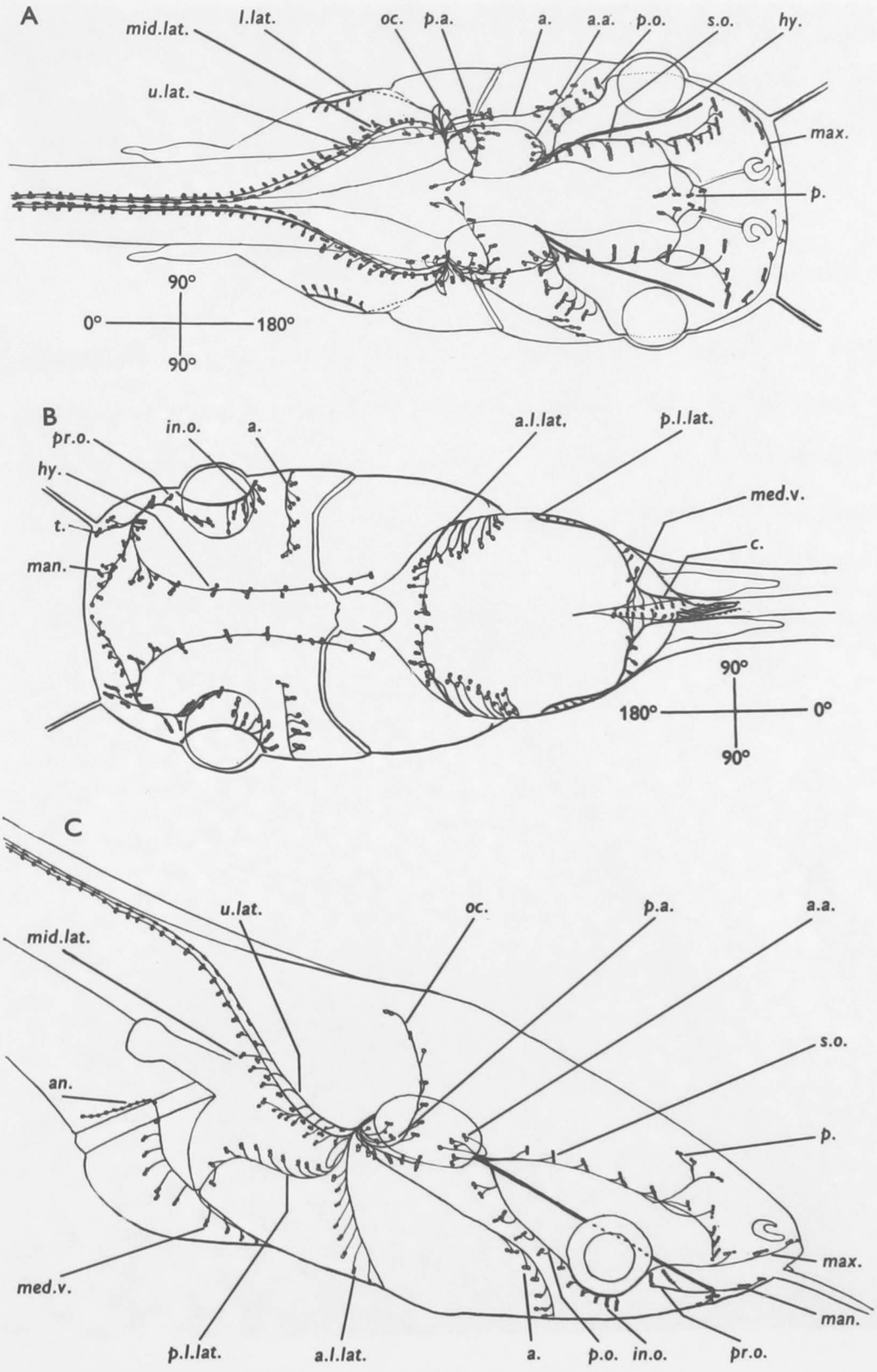
VII. & VIII.
a.l.
V.
IV.
B
a.l.
p.l.
C
p.l.
a.l.

Figure 3.

The distribution of sense organs in a stage 55 larva. (A) Dorsal view, (B) ventral view, (C) lateral view; a = aortic lateral line row; a.a. = anterior auditory lateral line group; a.l.lat. = anterior lower lateral line; an. = anal lateral line; c. = caudal lateral line; hy. = hyomandibular lateral line; in.o. = infra-orbital lateral line; l.lat. = lower lateral line; man. = mandibular lateral line; max. = maxillary lateral line; med.v. = median ventral lateral line; mid.lat. = middle lateral line; oc. = occipital lateral line; p. = parietal lateral line; p.a. = posterior auditory group; p.l.lat. = posterior lower lateral line; p.o. = post-orbital lateral line; pr.o. = pre-orbital lateral line; s.o. = supra-orbital lateral line; t. = tentacular lateral line group; u.lat. = upper lateral line.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s.o.
an.
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max.
med.v.
man.
p.l.lat.
a.l.lat.
a.
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in.o.
pr.o.

Figure 4.

This whole mount skin preparation from a stage 54 tadpole shows three lateral line plaques from the mandibular line. Each plaque is innervated by a bundle of nerves, these branch to supply every organ in the plaque. The skin was fixed and stained with osmium tetroxide.

Scale 50 μm.

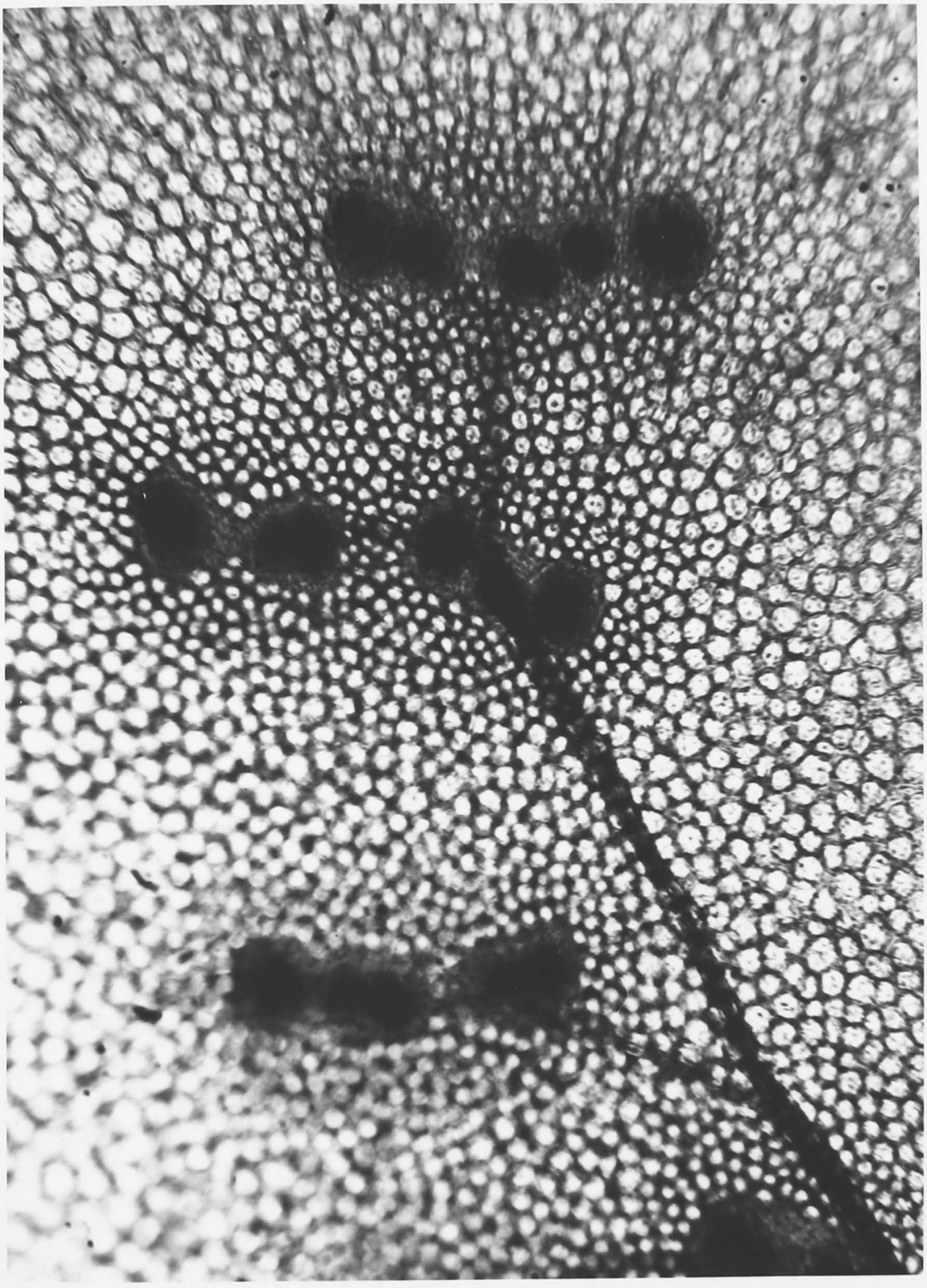

Figure 5.

(A) Two lateral line organs from the supra-orbital row in a stage 54 tadpole seen in a whole mount preparation of skin. The main nerve trunk can be seen running from left to right beneath the organs (arrows). The surface of the organs projects well above the surface of the skin.

Scale 50 μm.

(B) A tadpole organ at stage 54 seen in cross section to show sensory hair bundles (h) and dense staining of certain of the receptor cells. The preparation is a thin araldite section stained with toluidine blue.

Scale 5 μm.

(C) A section through the sensory hair bundle of a single receptor cell to show the single kinocilium (k) to one side of a group of stereocilia (st). Microvilli (mv) are also visible.

Scale 0.5 μm.

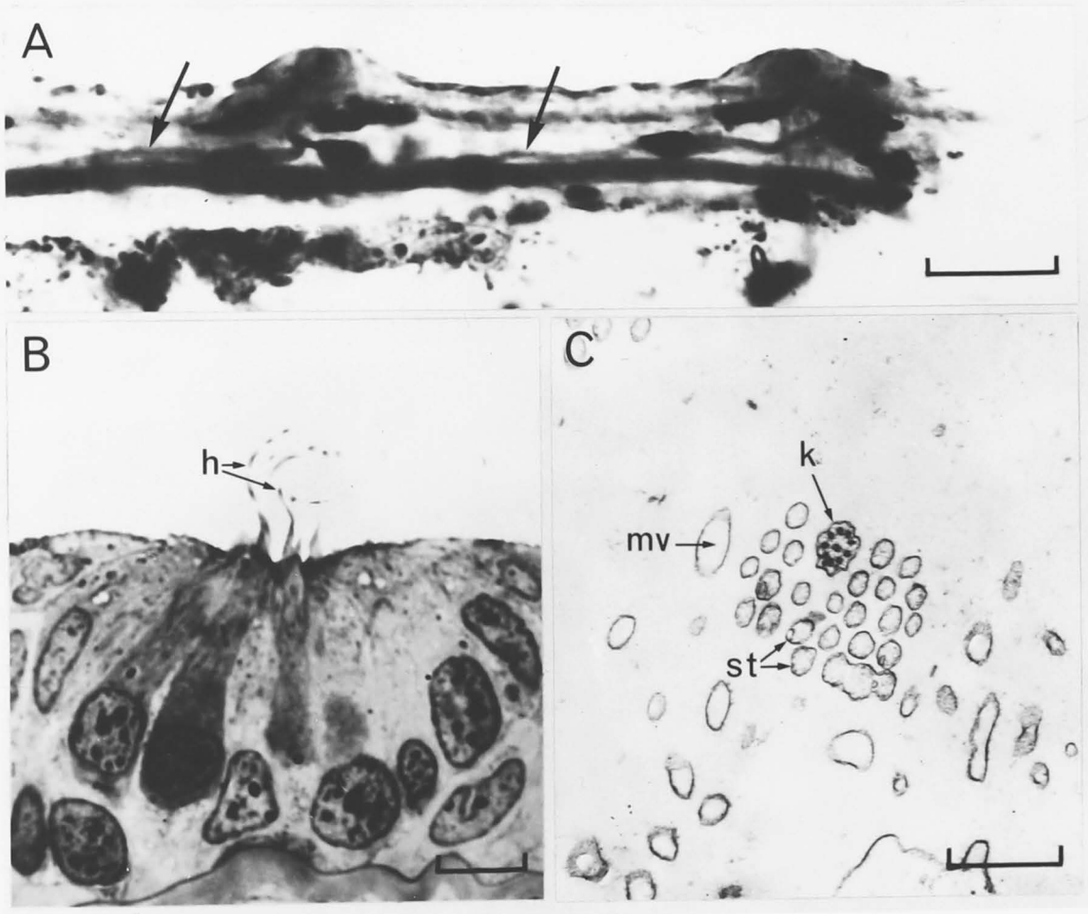
A
B
h
C
k
mv
st

Figure 6.

This is an electron micrograph showing a longitudinal section through a single lateral line organ from the supra-orbital row of a stage 56 tadpole. Mantle cells (m.c.) surround the organ and separate the receptor cells (r.c.) from the epidermal cells (e.p.). The receptor cells are also isolated from the basement lamella (b.l.) by basal cells (b.c.). There are fairly large intercellular spaces (i.s.) around the organ.

The receptor cells are pear shaped with nerve terminals (n) at their bases. Sensory hairs of two types, kinocilia (k) and stereocilia (st) project from the apical cuticular plate (c.p.) region of the receptor cells. Dark receptor cells (d.r.c.) are often present in the tadpole lateral line organs.

Scale 1.0 μm.

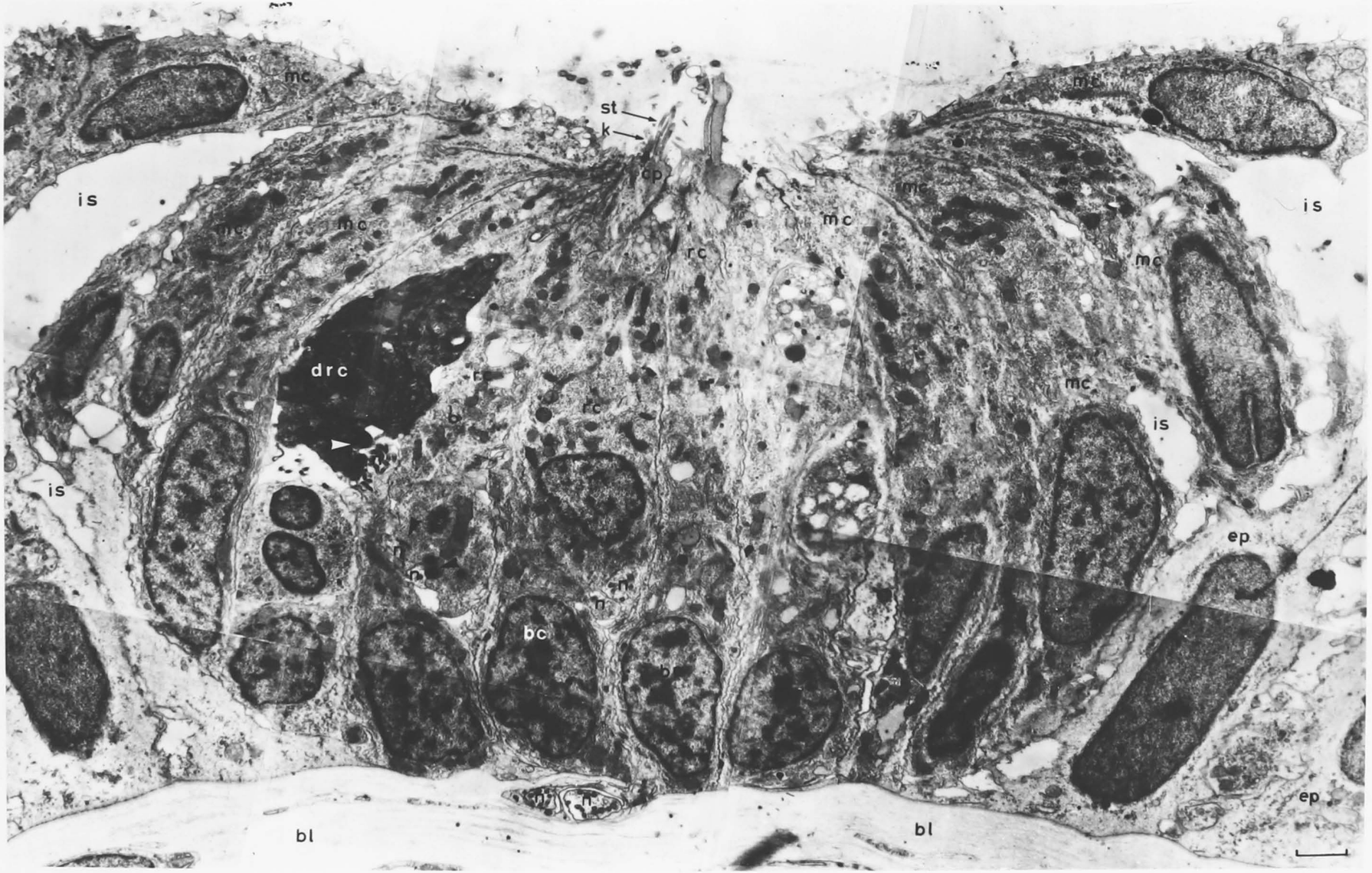

Fig 6

Figure 7.

These electron micrographs reveal details of receptor cell structure.

(A) Longitudinal section through a receptor cell (r.c.) shows how it tapers towards the apical region and the nucleus is situated in the lower third of the cell. A single kinocilium (k) and a group of stereocilia (st.) emanate from the peripheral end of the cell, the latter having their rootlets in a densely staining cuticular plate (c.p.). Nerve endings (n.e.) contact the receptor cell base.

(B) and (C) Details of the ciliary processes from two receptor cells. The stereocilia nearest to the kinocilium are longer than those on the far side of the cell where they may be only 0.4 μm long. The basal body of the kinocilium is placed to one side of the cuticular plate and its basal foot (arrowed) points away from the stereocilia.

(D) The section shows Golgi complexes (G) in a receptor cell. The region of the receptor cell above the nucleus is packed with elongated mitochondria, vesicles and endoplasmic reticulum.

(E) Beneath the nucleus the mitochondria are much larger and more spherical in shape than those above the nucleus.

(F) Two types of nerve ending (n.e.) are found at the bases of receptor cells and both types are represented in this figure. One type (the left hand ending) is characterised by the presence of many vesicles while the other has relatively few vesicles. More specific details are shown in subsequent micrographs. s.v. = synaptic vesicles in a receptor cell.

Scales: A 1.0 μm D 0.5 μm

B 0.3 μm E 0.5 μm

C 0.2 μm F 0.5 μm

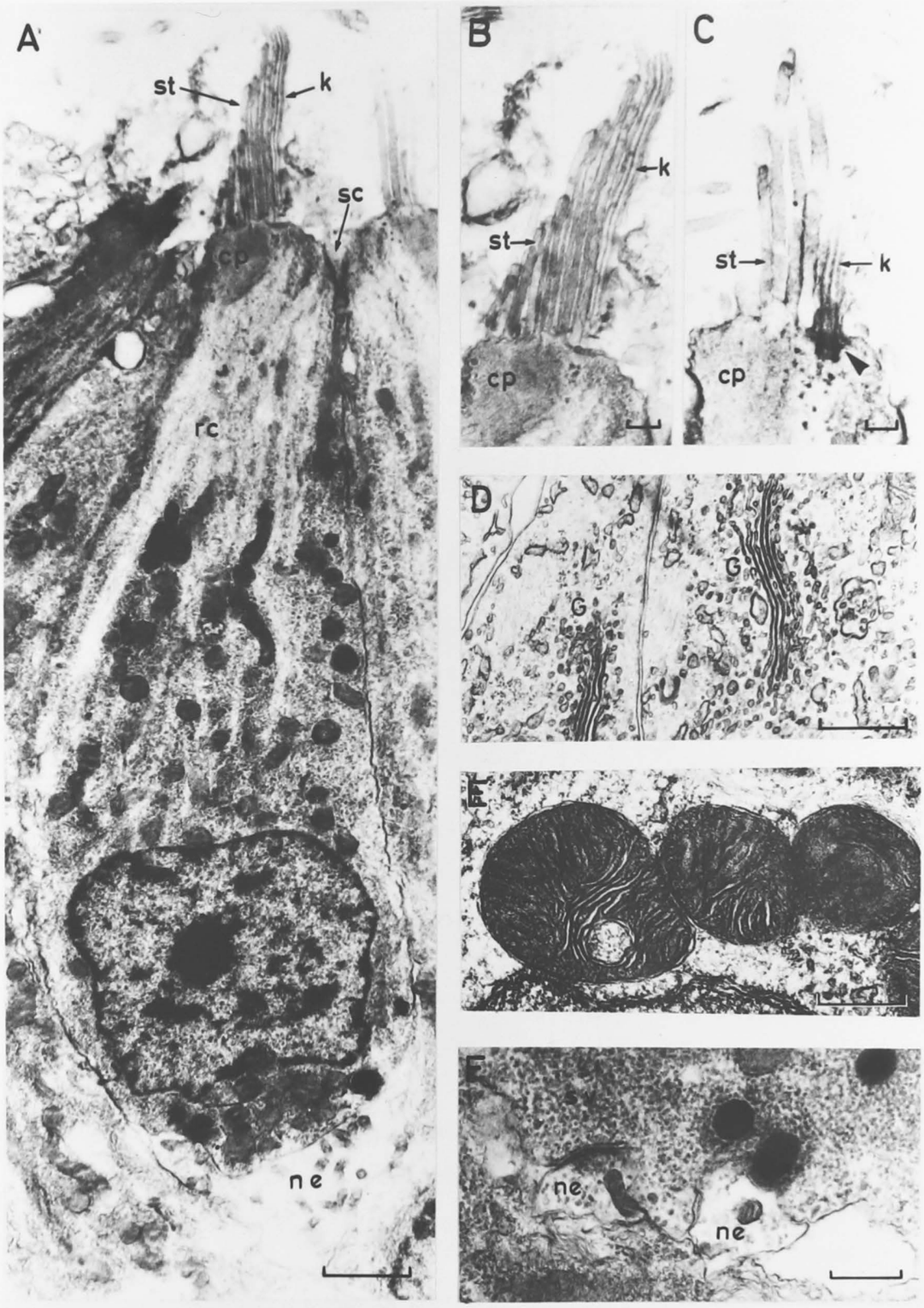
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Figure 8.

This electron micrograph shows the stereocilia attached to the cuticular plate (cp) of a receptor cell. Fibrils (f) can be seen within the stereocilia, they run together to form a dense rootlet (r) where the stereocilia are joined to the cuticular plate. Vesicles (v) between the stereocilia may represent material from the cupula. Also visible is a glancing section of a kinocilium (k).

Scale 0.2 μm.


k
f
f
v
r
cp

Figure 9.

Cytological detail of the upper region of a receptor cell is revealed in this electron micrograph. Prominent are the microtubules (mt) which run from the lower margin of the cuticular plate (cp). Mitochondria (m) in this region are elongate and are orientated along the long axis of the cell. Multivesiculated bodies (mvb) are common but their function is unknown. Basal bodies (b) or centrioles are found in the tops of all receptor cells.
Scale 1.0 μm.

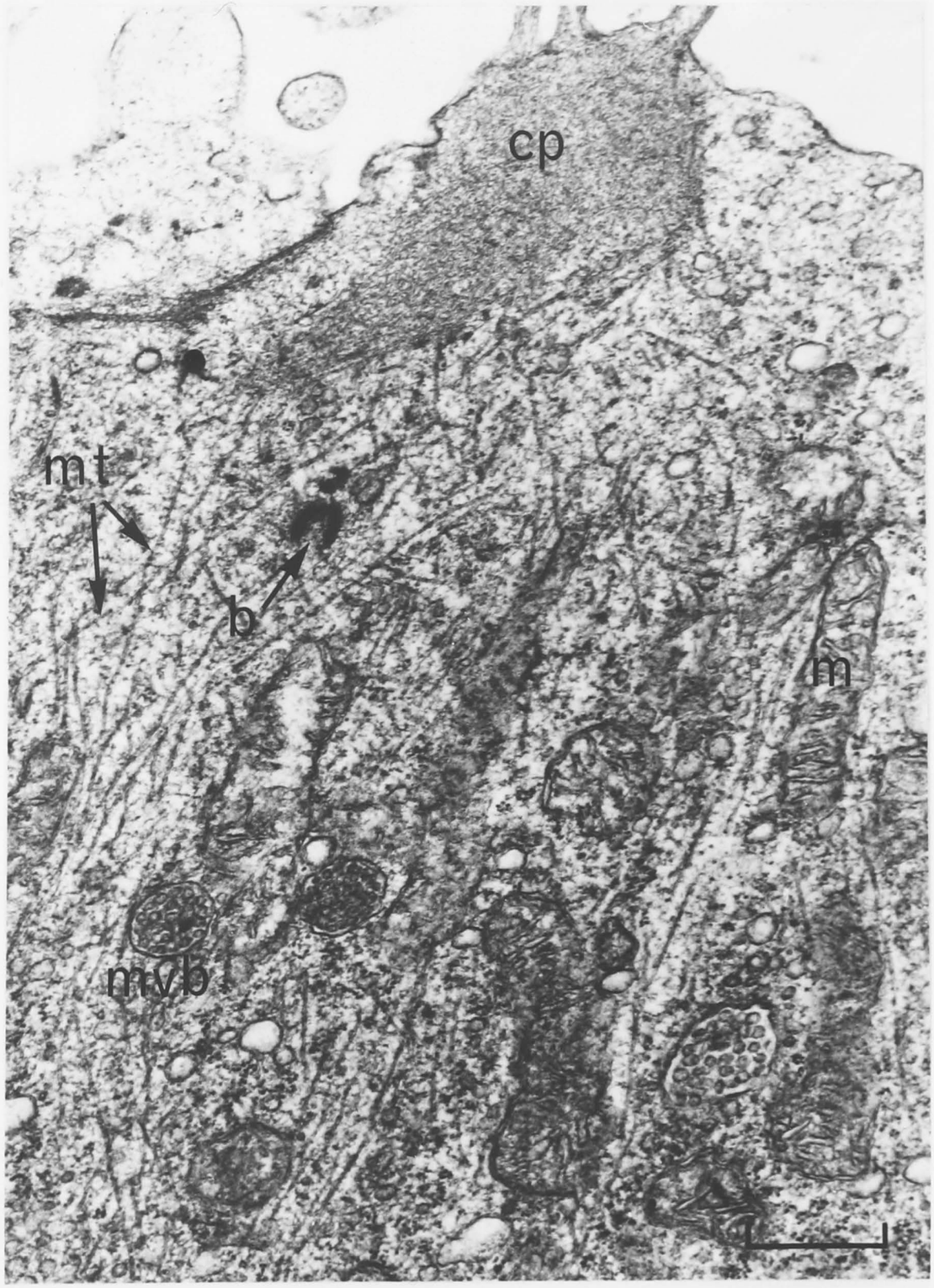

cp
mt
b
m
mvb

Figure 10.

This micrograph reveals cytological detail of the tops of receptor cells and the hair-cell bundles. The upper regions of receptor cells are attached to supporting cells by desmosomes (d). Each hair-cell bundle consists of two types of projection, the stereocilia (st) and a single kinocilium (k). The stereocilia have their rootlets (arrows) in the densely staining cuticular plate (cp) region of the receptor cell. Between the stereocilia amorphous material (cs) may represent the cupula substance. Also visible in the micrograph are microvilli (mv) from the tops of supporting cells and centrioles (c) in the region below the cuticular plate of the receptor cells.

Scale 1.0 μm.

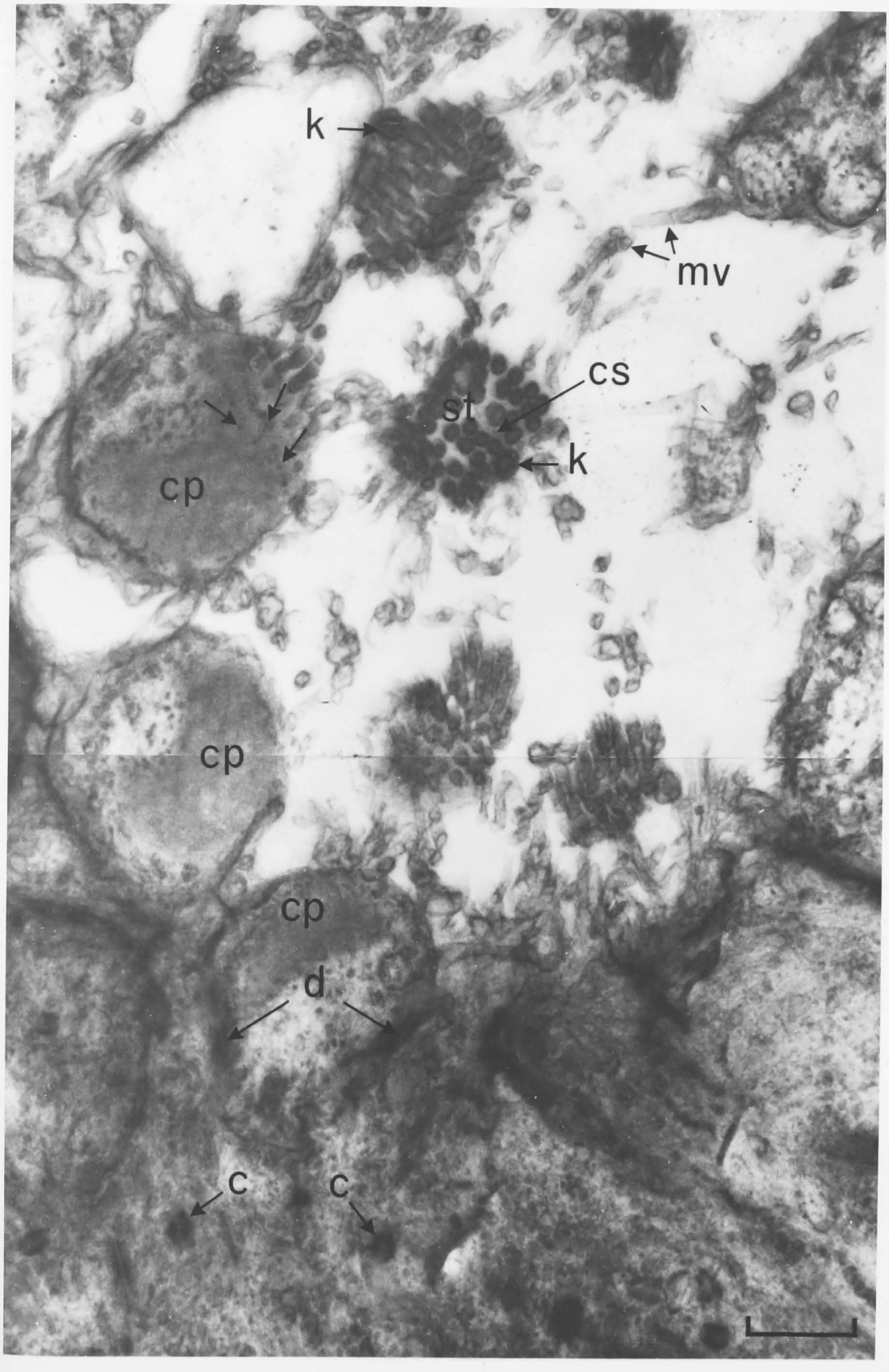

k
mv
cs
st
k
cp
cp
cp
d
c
c

Figure 11.

The electron micrograph shows a densely staining receptor cell (dark receptor cell) (d.r.c.) from a larval organ. Normal receptor cells appear on the right and supporting cells (s.c.) on the left. The inset shows the upper part of the cell with the cuticular plate (c.p.), a kinocilium (k) with a basal body (b) and processes (p) from the cuticular plate. Normal stereocilia are absent and the kinocilium has the same electron-density as the main part of the receptor cell.

Scales: Main micrograph 1.0 μm

Inset 0.5 μm

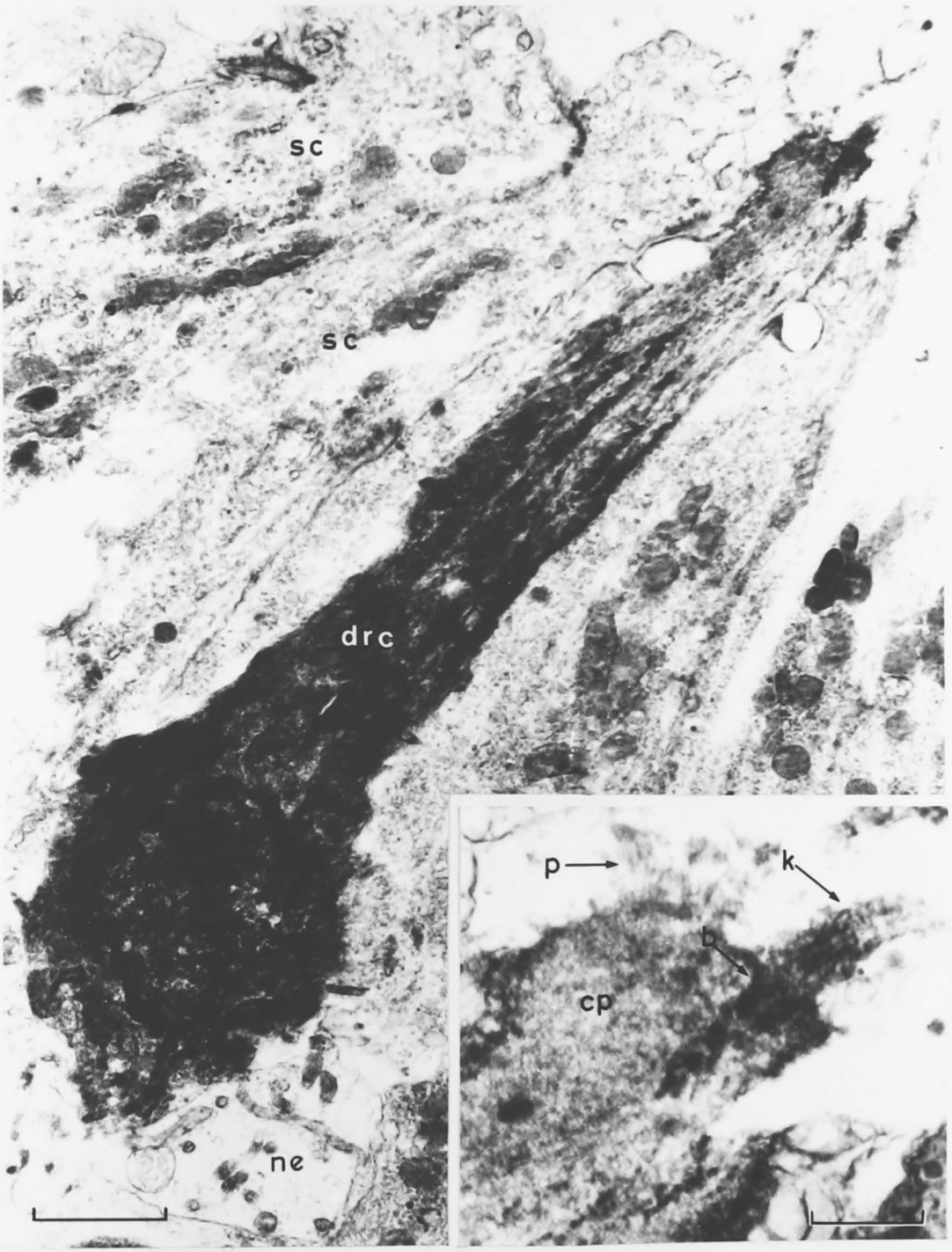

sc
sc
drc
ne
p
k
b
cp

Figure 12.

Certain of the receptor cells stain densely with toluidine blue. Electron micrographs show that the cytoplasm is packed with organelles and that the nuclear material is clumped in irregular masses.

(A) This receptor cell has enlarged cysternae of the endoplasmic reticulum (c) and typical dense cytoplasm. A non-granulated nerve ending (n.g.) is closely associated with the receptor cell.

(B) This cell has clumped nuclear material (N) and is typical of densely staining cells.

(C) The cytoplasm often contains myeloid bodies (my.) and membrane complexes. The contrasting electron density of this and the surrounding cells is very distinct.

(D) This micrograph shows elaborate mitochondria (m.) typical of dense receptor cells. They maintain connections with the non-granulated endings (n.g.) and the inset shows an enlarged view of the synaptic contact with one such ending. Arrows show characteristic membrane modifications.

Scales A 1.0 μm

B 0.5 μm

C 1.0 μm

D 1.0 μm

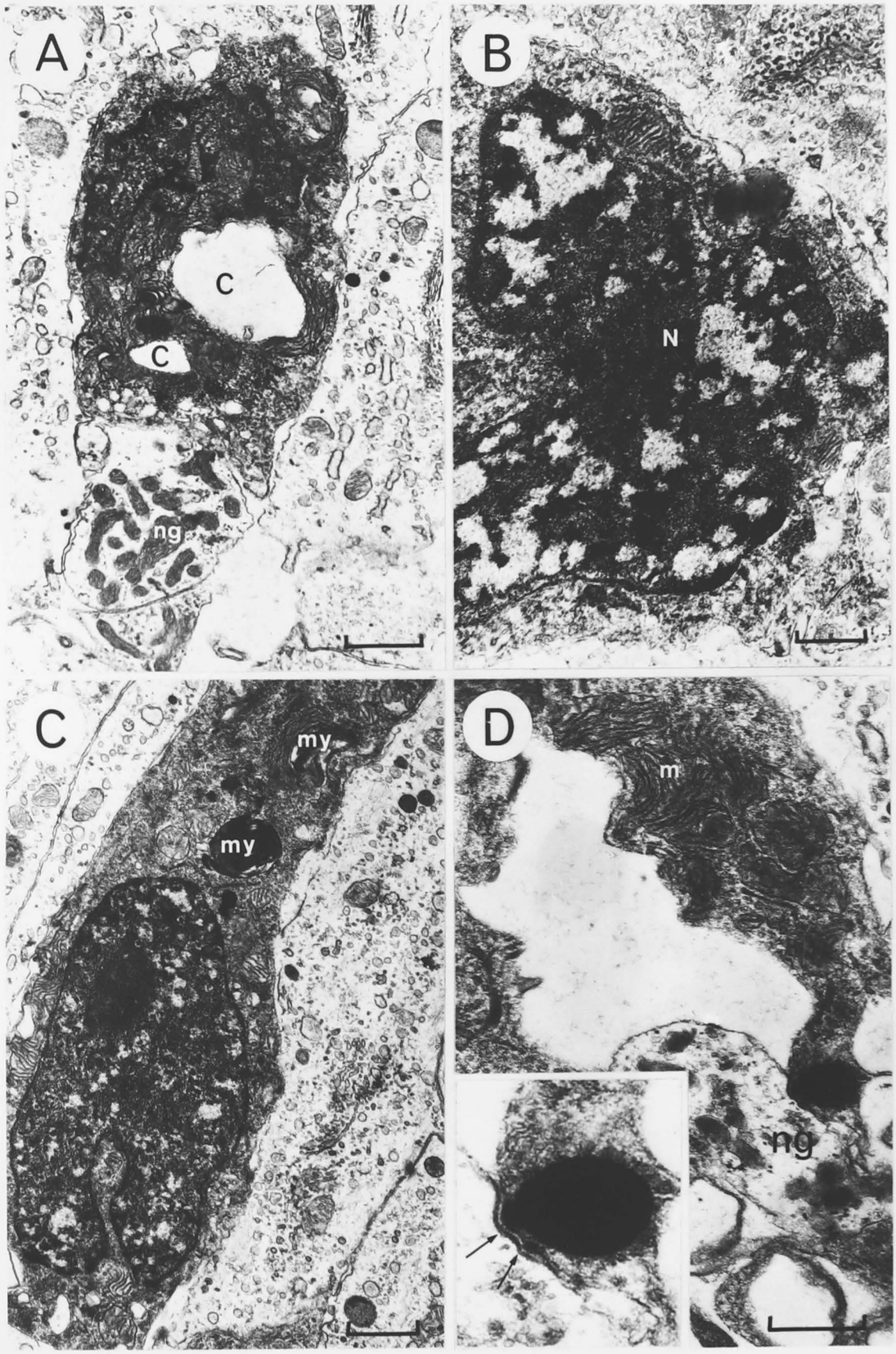

A
C
C
ng
B
N
C
my
my
D
m
ng

Figure 13.

These four figures are selected from serial electronmicrographs of the top of a larval organ. The complete series of 20 sections was used to determine the arrangement of hair-cell bundles in one organ. Seven bundles can be distinguished in (A), two of them have their kinocilium nearer to the top of the page and five have the kinocilium nearer to the bottom. Micrographs B, C and D show the same organ at successively lower levels. The electron dense cuticular plates of receptor cells can be distinguished in all four figures. In D, dense membranes (arrows) indicate the desmosomes which connect receptor cells and adjacent supporting cells. Numbers at the bottom right of each section indicate the position of each micrograph in the series.

Scales 1.0 μm.

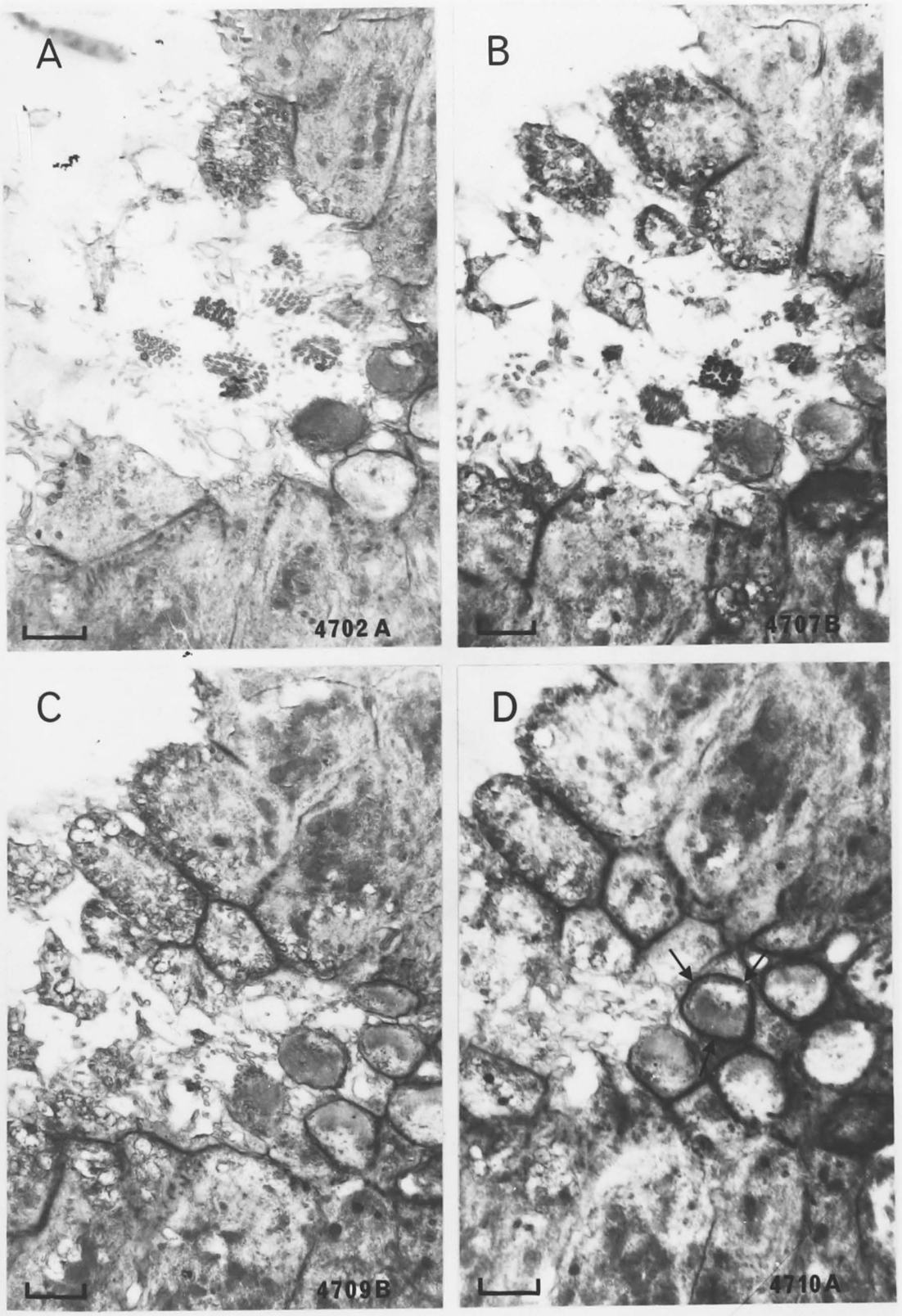

A
4702 A
B
4707B
C
4709B
D
4710A

Figure 14.

This diagram shows the arrangement of sensory hair bundles of all the receptor cells of one larval organ. Two classes of cell can be distinguished on the basis of the positions of their kinocilia with respect to the stereocilia. There are thirteen with the kinocilium nearer to the top of the page and nine with the kinocilium nearer to the bottom of the page. The line X-Y represents the mid-line and long axis of the organ plaque in which the organ was situated. The classes of receptor cell are intermingled and there is no separation of the two types about the mid-line. The stippled part of each cell represents the cuticular plate and the small circles the basal bodies of the kinocilia.

Scale 1 μm.

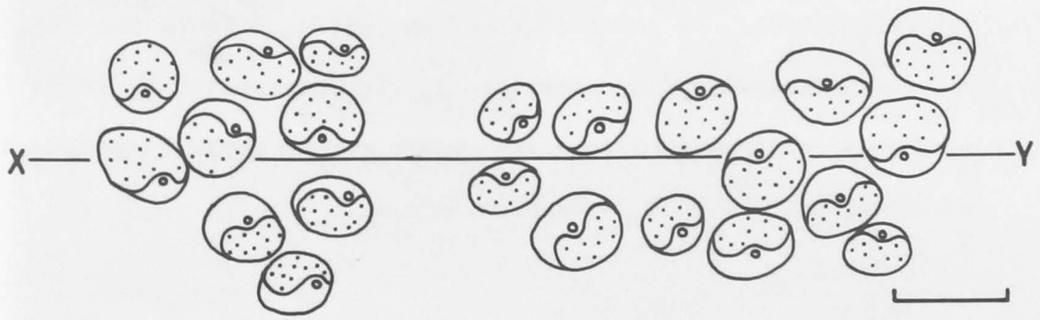
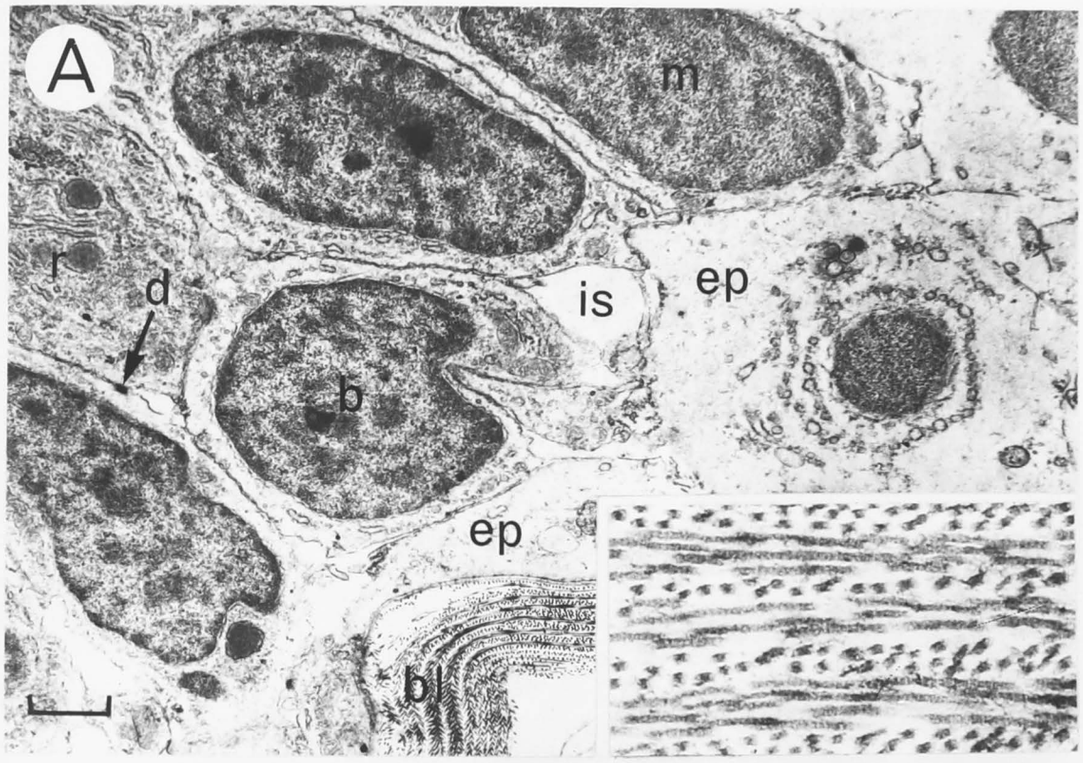

X
Y

Figure 15.

(A) Receptor cells (r) are separated from the epithelium (ep) surrounding the organ by basal (b) and mantle cells (m). They are also separated from the basement lamella (bl) by basal cells. The inset shows the layered fibrils of the basement lamella. A third class of supporting cell is the sustentacular cells (not shown here), they are small flattened cells which lie between the receptor cells.
d = desmosome; i.s. = intercellular space.
Scale 1.0 μm.

(B) Structural detail of the supporting cells is fairly similar for all classes. Visible in the cytoplasm of this basal cell are vesicles (v) of assorted sizes, mitochondria and Golgi complexes (G). n = nucleus.
Scale 1.0 μm.


A
m
r
d
is
ep
b
ep
bl

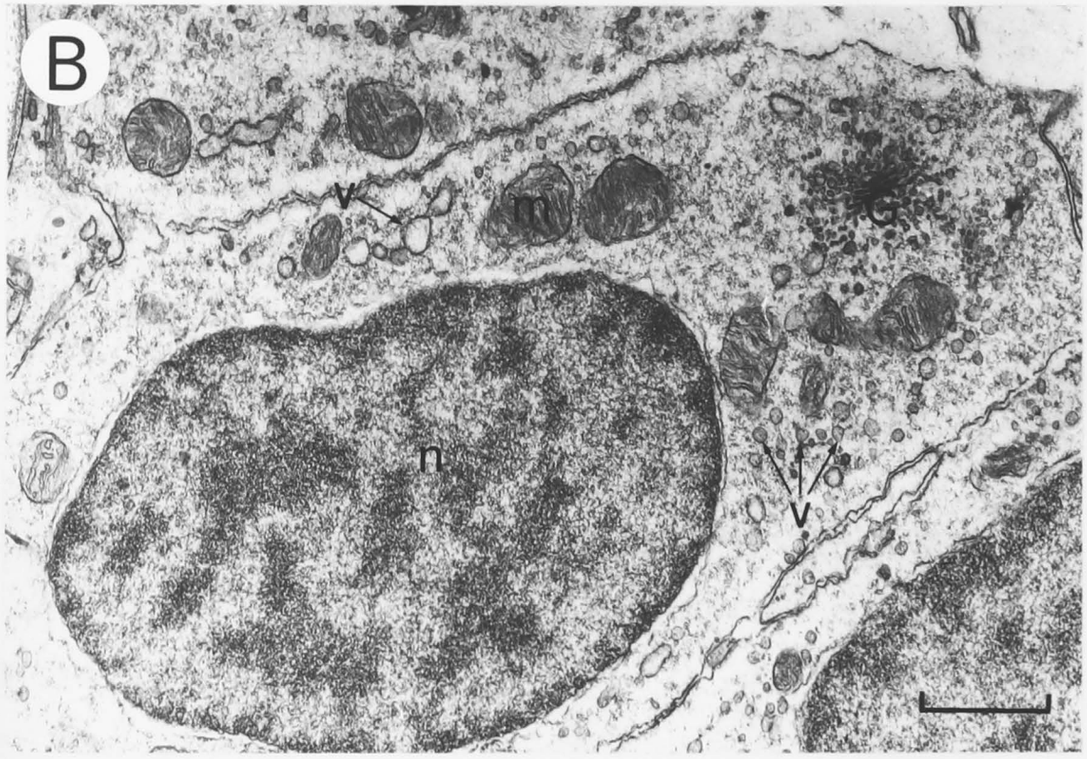

B
v
m
G
n
v

Figure 16.

These electron micrographs show the main cytological details of the lateral line nerve trunks at stage 54. There are two clearly distinguishable groups of fibres; those which are myelinated and those which are not. (A) There is a wide spectrum of myelinated fibre diameters, some are little bigger than the unmyelinated axons (um). Details of the two fibre types are shown in (B) and (C), the inset shows the characteristic myelin structure.

e.r. = endoplasmic reticulum; c. = collagen fibres; g. = glial elements.

Scales: A 2.0 μm

B 1.0 μm

C 0.5 μm

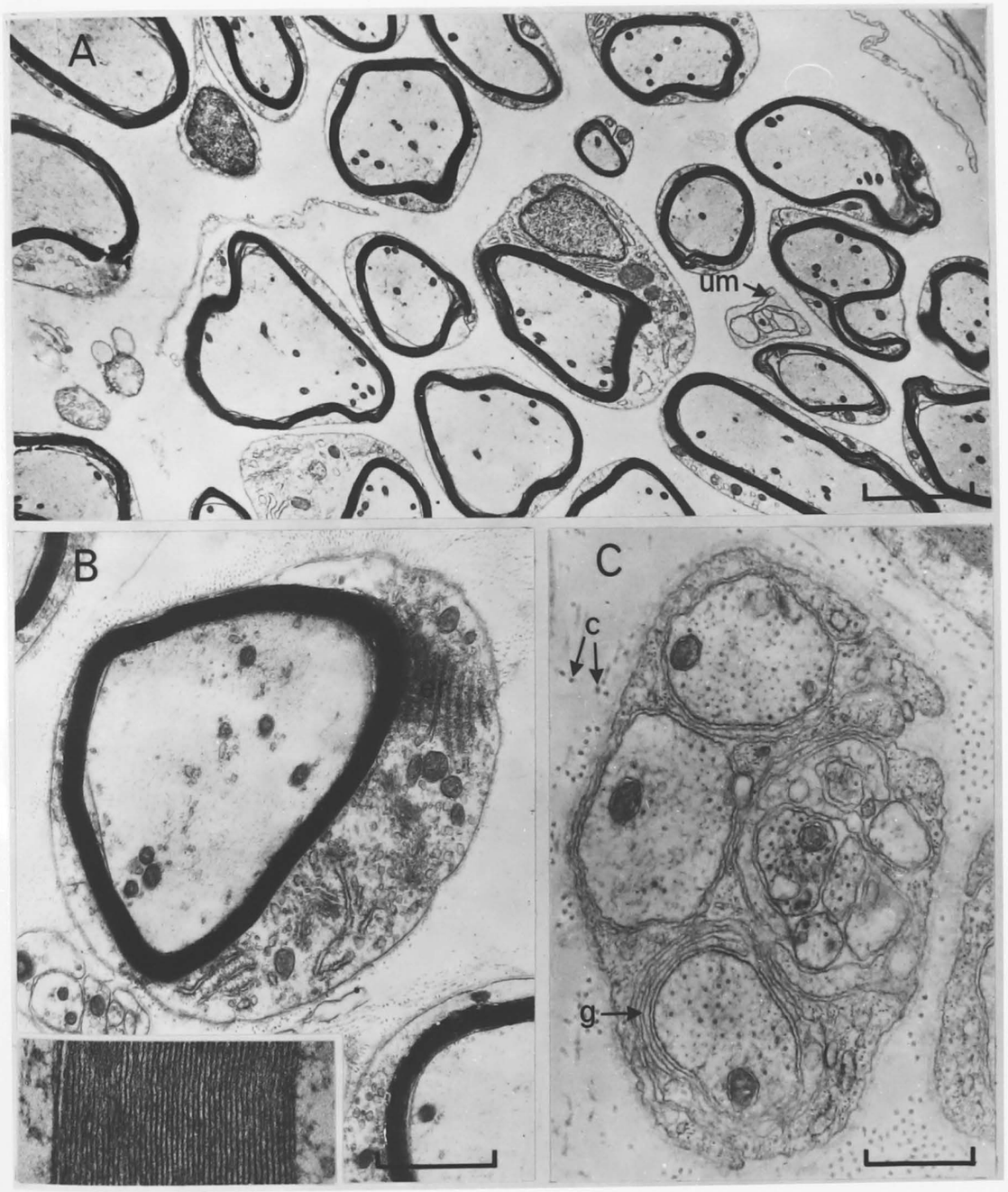

A
um
B
C
c
g

Figure 17.

These four micrographs of the innervations of different single plaques in stage 58 tadpoles show that each bundle consists of two myelinated fibres (a) and a bundle of unmyelinated ones surrounded by a glial element (g). The number of unmyelinated fibres is indicated in the bottom left hand corner of each micrograph.

Scales 1.0 μm.

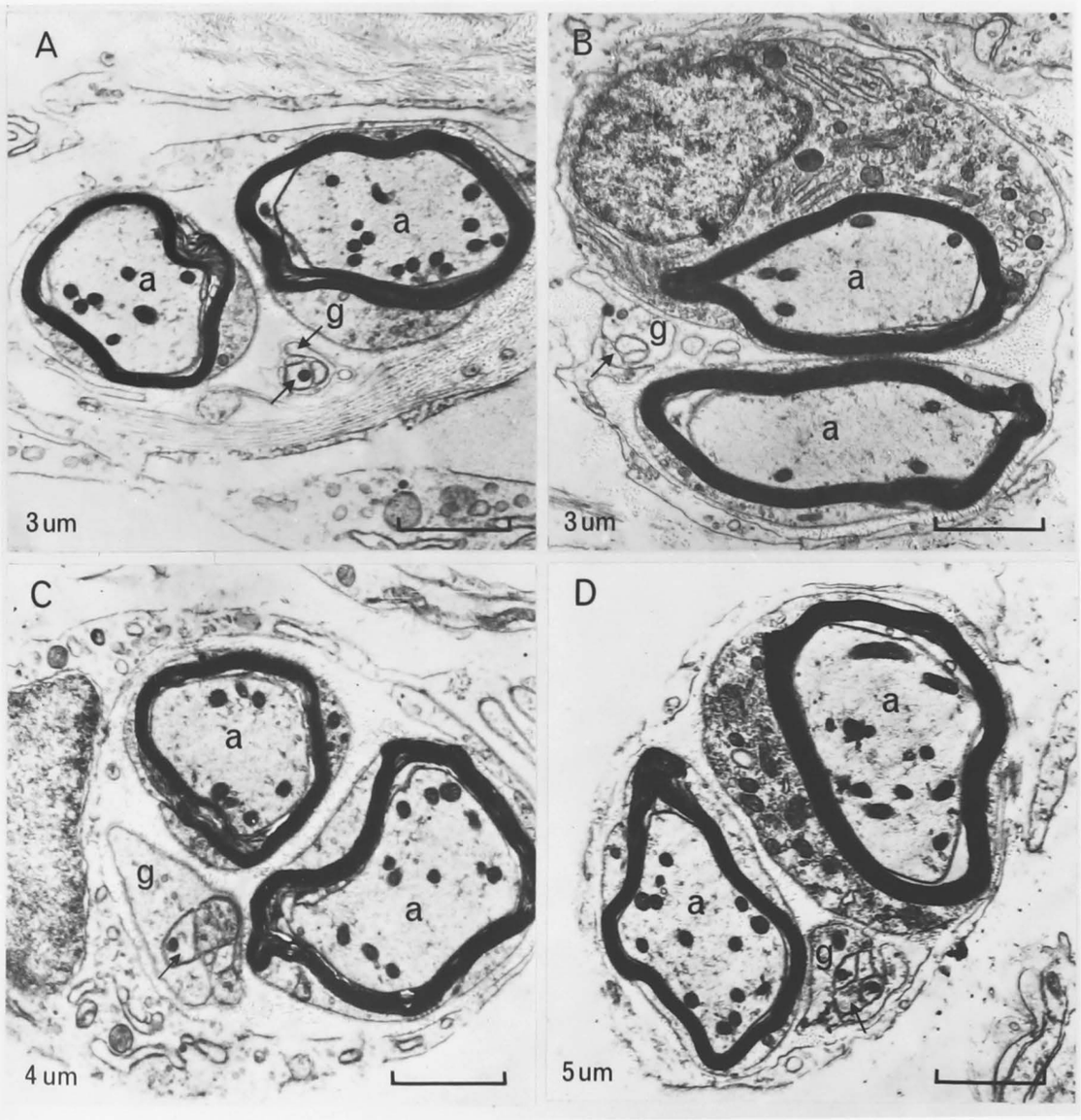

A
a
a
g
3um
B
a
g
a
3um
C
a
g
a
4um
D
a
a
g
5um

Figure 18.

(A) The nerve fibres (n) supplying each organ pass through the underlying basement lamella. The basement lamella consists of layers of collagen-like fibrils (c). Because the nerve fibres are all unmyelinated at this point different functional classes are not distinguishable.

Sch = Schwann cell.

(B) Nerve processes pass up between the basal cells (b.c.) en route for the receptor cells. Five unmyelinated nerves are visible in this micrograph.

(C) Nearer to their terminals at the bases of the receptor cells, granulated (g.n.) and non-granulated (n.g.) endings can be distinguished. Often the two types of ending are close to each other but specialised synaptic contacts between them have not been seen in random electron micrographs of this region.

(D) In this micrograph non-granulated terminals can be seen in contact with the receptor cells (r).

s = supporting cell.

Scales: A 1.0 μm

B 1.0 μm

C 0.5 μm

D 3.0 μm

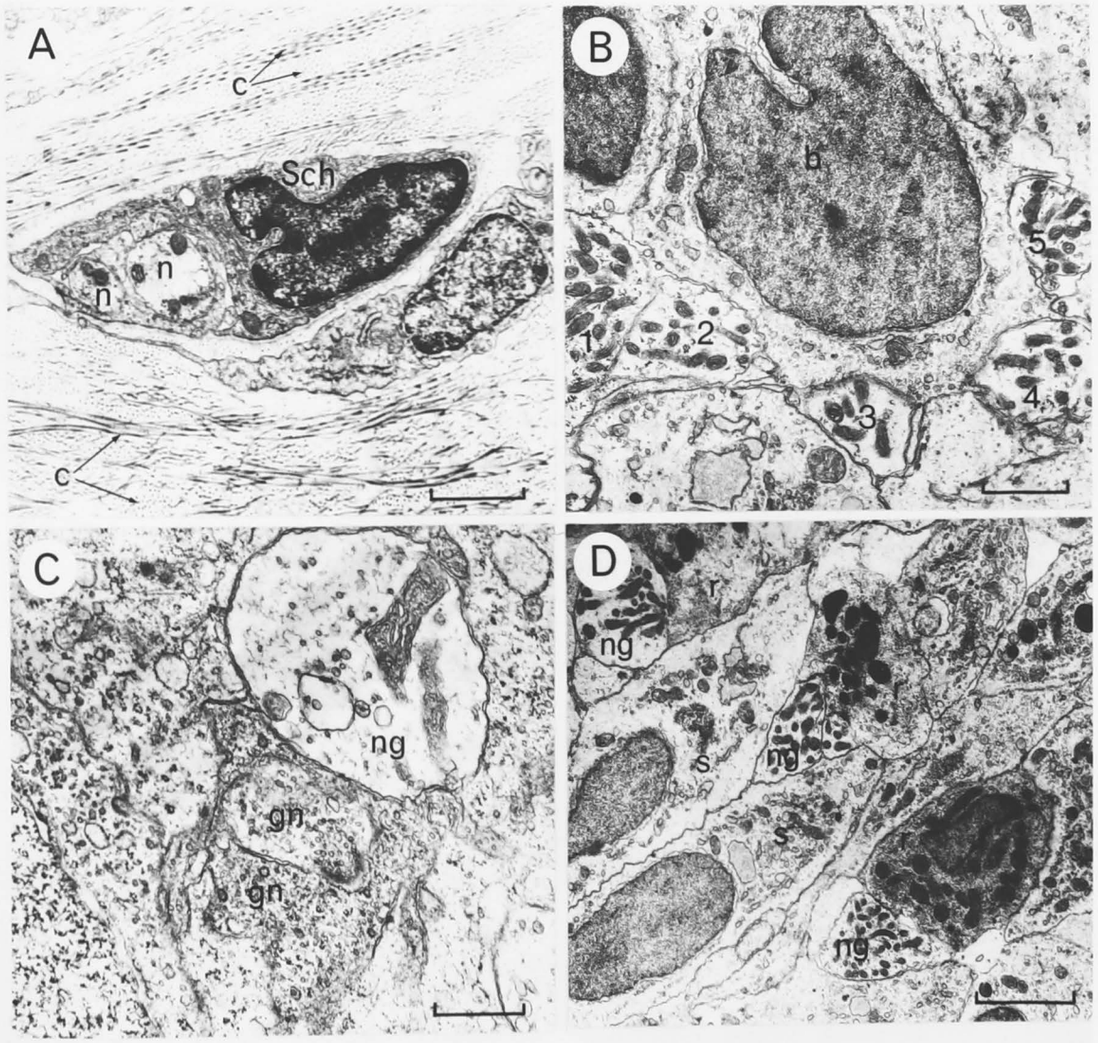

A
c
Sch
n
n
c
B
b
1
2
3
4
5
C
ng
gn
gn
D
r
ng
s
ng
s
ng

Figure 19.

(A) This electron micrograph shows two so-called granulated endings (GN) in contact with a receptor cell (R). This type of ending is characterised by the presence of densely packed synaptic vesicles and a few mitochondria. There is no densely staining body next to the receptor cell membrane at the point of contact. Other branches of the granulated ending type can be seen in the top right of the micrograph.

Scale 1.0 µm.

(B) A high power detail of the type of contact shown in (A). A uniform synaptic cleft can be seen and in the receptor cell a post-synaptic cistern can be distinguished.

Scale 0.5 µm.

(C) An electron micrograph showing a non-granulated ending (NG) in contact with a receptor cell (R). The densely staining synaptic bars (sb) often occur singly or in pairs lying next to the receptor cell membrane. This is thickened near to the bars.

Scale 0.75 µm.

(D) A higher power micrograph of the contact of a non-granulated (NG) fibre with a receptor cell (R). The membrane of the receptor cell is clearly modified and the synaptic cleft (sc) appears to contain amorphous material. There are few vesicles in the nerve ending.

Scale 0.1 µm.

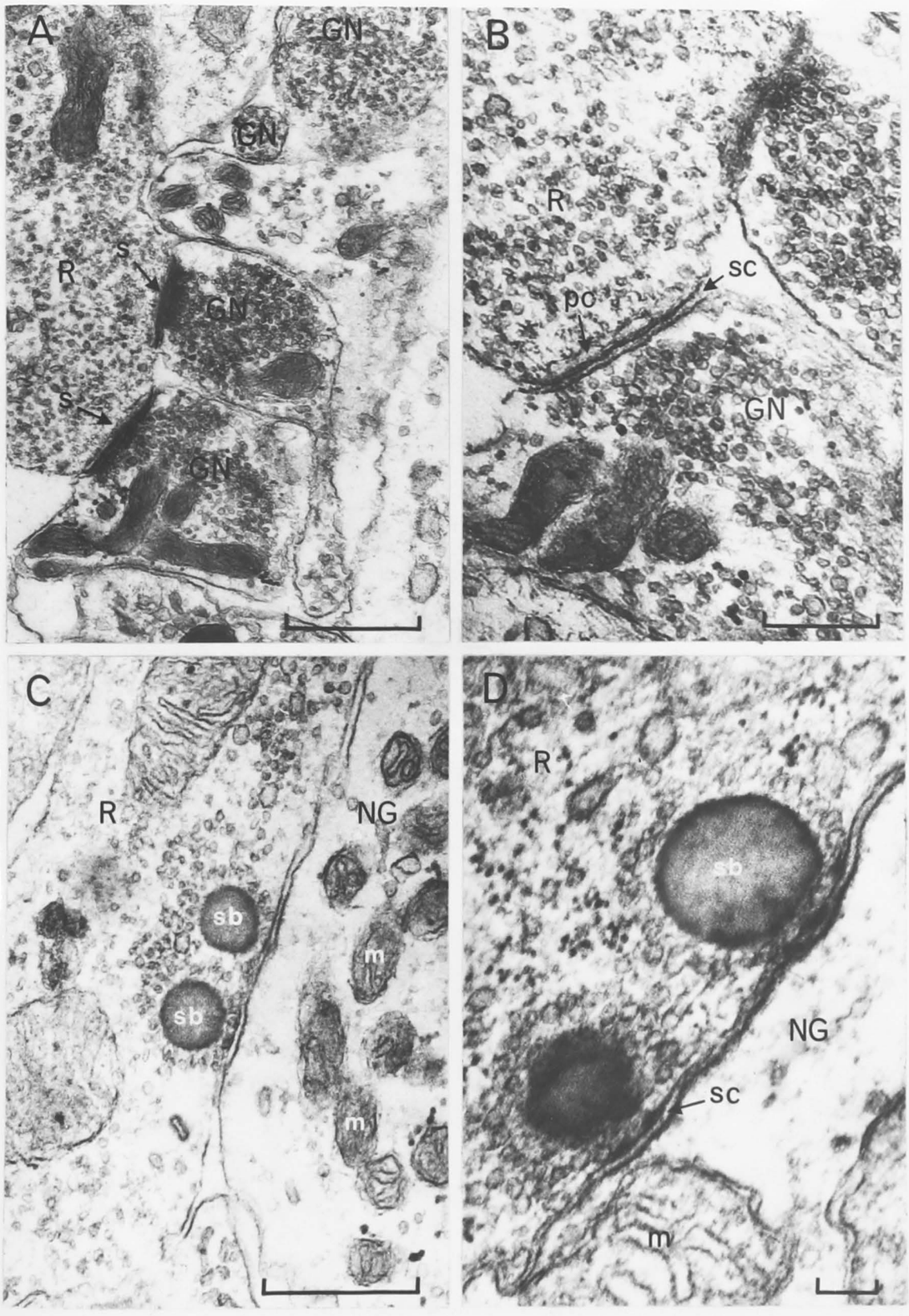

A
GN
GN
R
S
GN
S
GN
B
R
sc
pc
GN
C
R
NG
sb
sb
m
m
D
R
sb
NG
sc
m

Figure 20.

(A) A densely-staining process between two sensory cells (SE) could be a third type of nerve ending (NE 3). This type of structure is referred to as an 'electron dense ending' in the labyrinth of the Thornback ray (Lowenstein et al 1964). Its occurrence has not been previously recorded in the lateral line system. Scale 0.25 μm.

(B) This detail from Lowenstein et al (1964) shows an electron dense ending situated in a small depression on the surface of a sensory cell (SE). Also visible is a non-granular type of ending (NE 1) encapsulated by the plasma membrane of the sensory cell. This figure is included for comparison with the newly described electron dense ending of the lateral line system shown above.

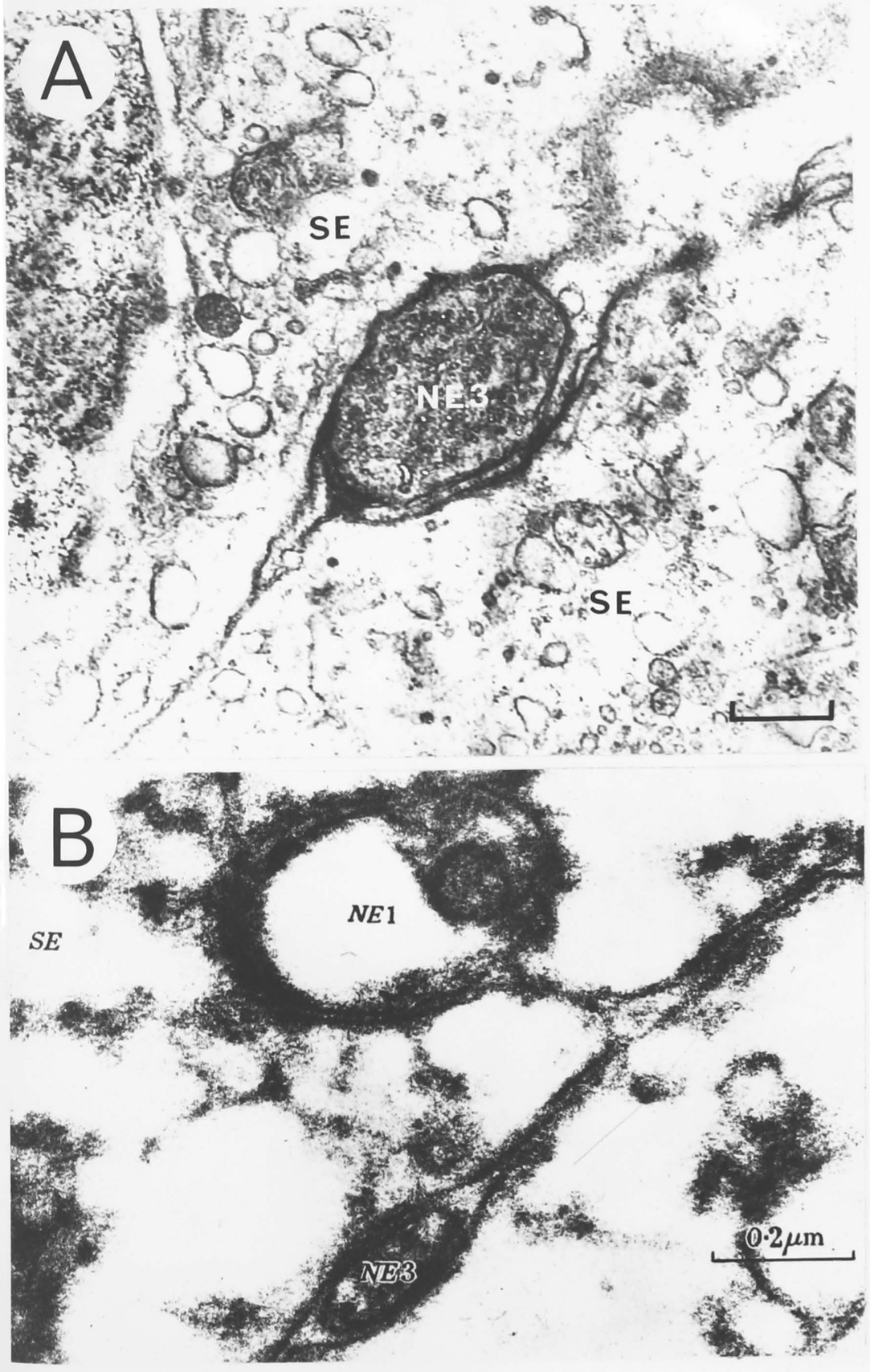
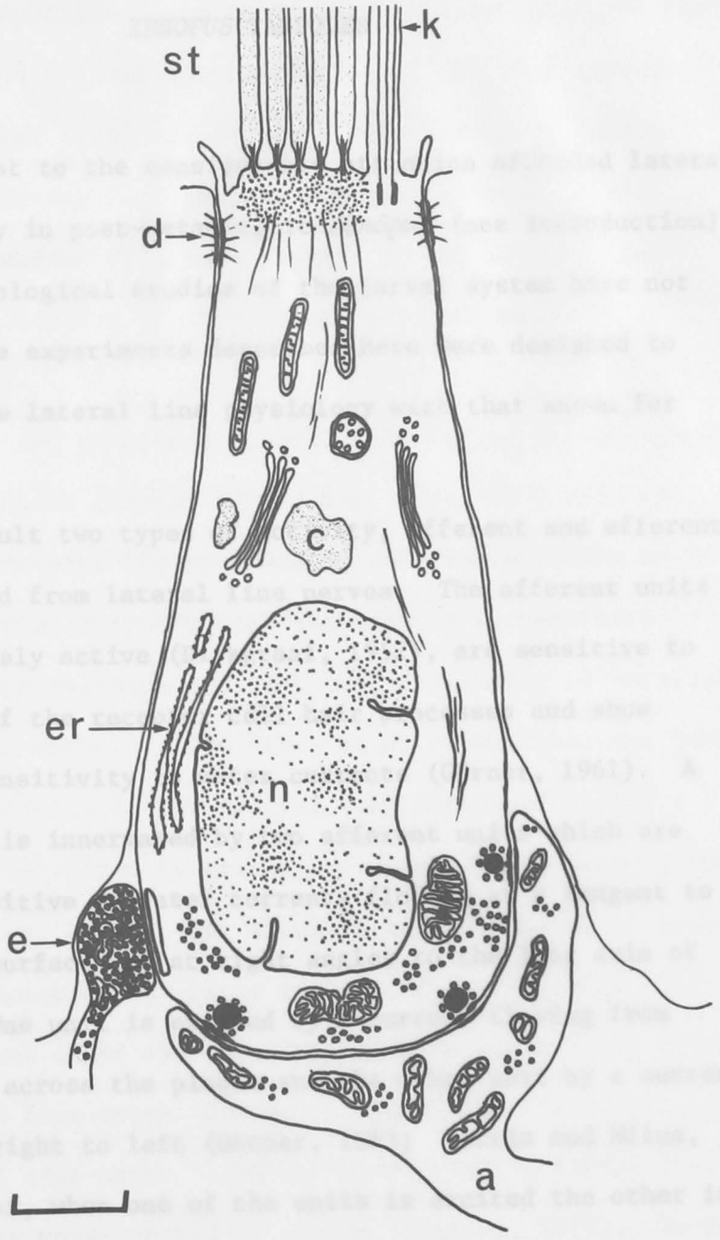

A
SE
NE3
SE
B
NE1
SE
NE3
0·2μm

Figure 21.

The fine structure of the tadpole receptor cells is summarised in this figure. The presumed afferent endings (A) form a cup around the base of the cell, there are many specialised synaptic sites between the cell and the nerve ending. The so-called efferent endings (E) are much smaller, there may be several such endings per cell. Electron dense endings are not shown in this diagram.

K = Kinocilium; ST = Stereocilia.

Scale 1 μm.


k
st
d
c
er
n
e
a

CHAPTER IV

EXTRACELLULAR RECORDING FROM THE LATERAL LINE NERVES OF *XENOPUS* TADPOLES

In contrast to the considerable attention afforded lateral line physiology in post-metamorphic *Xenopus* (see Introduction) previous physiological studies of the larval system have not been made. The experiments described here were designed to compare tadpole lateral line physiology with that known for the adult.

In the adult two types of activity, afferent and efferent, can be recorded from lateral line nerves. The afferent units are spontaneously active (Dijkgraaf, 1952), are sensitive to displacement of the receptor cell hair processes and show directional sensitivity to water currents (Görner, 1961). A single plaque is innervated by two afferent units which are maximally sensitive to water currents flowing at a tangent to the animal's surface and at right angles to the long axis of the plaque. One unit is excited by a current flowing from left to right across the plaque and the other unit by a current flowing from right to left (Görner, 1963; Harris and Milne, 1966). Further, when one of the units is excited the other is inhibited (Görner, 1963). Environmental parameters such as temperature (Murray, 1956) and the ionic composition of the water (Onada, Hashimoto and Katsuki, 1970) can influence the level of spontaneous activity. These parameters presumably do

not alter the directionality of the units but could impair the ability of the lateral line system to discriminate small stimuli.

The efferent units are not spontaneously active but are stimulated by ipsilateral or contralateral stimulation of the lateral line and by tactile stimulation of the skin (Görner, 1967). Prolonged bursts of efferent activity occur during movements of the legs and body of the toad (Görner, 1967). Further, spontaneous afferent activity in the lateral line nerve can be inhibited by stimulating efferent units (Russell, 1968).

There are two published reports relating to physiological activity in tadpole lateral line nerve. Schmidt (1965) was unable to demonstrate the presence of efferent activity in *Rana pipiens* lateral line nerves although he successfully recorded one labyrinthine efferent unit responding to horizontal rotation of the tadpole. The other study showed that the level of afferent activity in the lateral line nerves of larval *Xenopus* could be raised by placing a vibrating rod near to the tadpole (Shelton, 1970). The afferent units recorded in adults have been associated with large myelinated axons (thick fibres) and the efferent units with small myelinated axons (thin fibres) in the lateral line nerve (Görner, 1963). The thin fibres appear in the lateral line nerve at metamorphosis and in larval stages they are either unmyelinated or absent (Shelton, 1970). It is important to know whether or not an inhibitory system is present in the tadpole.

MATERIALS AND METHODS

(I) The recording chamber

The preparation and recording electrodes were enclosed in a humidity chamber made from perspex and lined with wet blotting paper. It accommodated a small dish containing a tadpole under saline (Fig. 22). Electrodes were introduced into the experimental chamber through holes in the lid. The atmosphere inside the chamber was allowed to saturate with moisture before the nerves of the dissected tadpole were lifted above the level of the saline. Part of the lid was removeable to facilitate manipulation, especially necessary when lifting nerves onto extracellular electrodes. This operation was carried out rapidly with the aid of small glass hooks made from fine bore capillary tubing. Using this arrangement, activity could be recorded from small efferent units for periods of at least 15 minutes. An alternative practice for preventing nerves from drying out is to lift them into an insulating layer of 'Boots' paraffin oil. Although this method was used initially nerves became physically hardened by the oil and units deteriorated rapidly. The humidity chamber was used for this reason.

(II) Recording arrangement

Various diameters (0.01 - 0.1 mm) of silver wire were used to make paired hook electrodes for differential recording from the nerves. Impulses were channelled through a Tetronix Type 122 Low-Level differential preamplifiers (pass-band 100-1,000 Hz) and displayed

on a Tetronix 502A dual beam oscilloscope. Oscillographs were recorded on moving photographic paper (Ilford NS6) and simultaneously recorded on tape using a Ferrograph Series Seven recorder. Nerves were stimulated electrically through silver wire hook electrodes using a Tetronix 160 Series pulse generator. The stimulus was isolated from earth using a stimulus isolation unit or a one-to-one transformer (Fig. 23). The arrangement of the apparatus is shown in Figure 24.

(III) Mechanical stimulation

The organs were stimulated mechanically by controlled jets of water, by drops falling onto the water surface and by means of a small vibrating tube. The tube was mounted on the arm of an Advance Type VI oscillator which was transformer coupled to an Advance AF sine wave generator HIE. The vibration of the water column enclosed by the tube was the effective stimulus. Stimuli were monitored on the lower beam of the oscilloscope. Opening the tap of the water jet closed a switch causing a DC deflection of the beam and similarly falling drops completed a circuit as they hit the water surface. A direct output from the waveform generator was used to monitor movements of the vibrating tube.

For all experiments the tadpoles were first anaesthetized with MS 222 and dissected prior to placement in the chamber (see Chapter II).

RESULTS

(I) Afferent activity

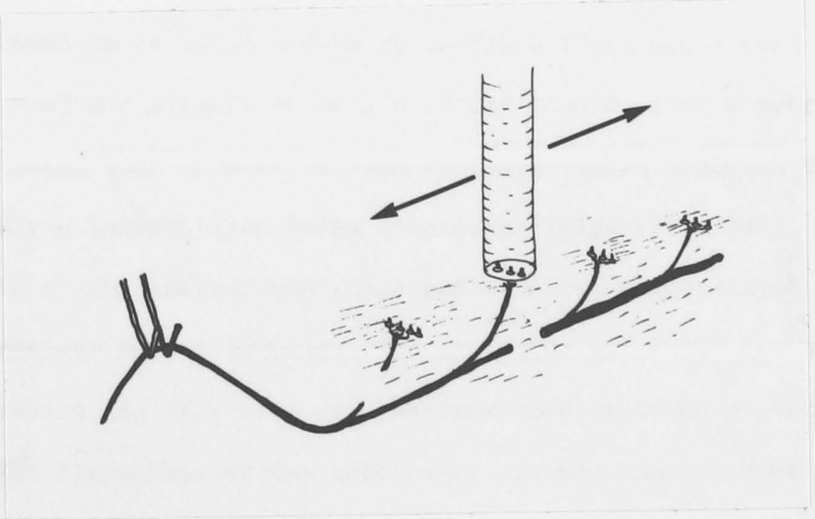
All electrophysiological recording of afferent units was made from the middle lateral line nerve which was cut centrally to the recording site to eliminate efferent activity. Lateral line plaques were stimulated either using a vibrating tube placed over the organs or by small water currents. A plaque consists of several organs innervated by branches of the same sensory nerves (see Chapter III). Some records were obtained from single plaques but most were from groups because it was difficult to isolate single plaques in so small a preparation. Recordings were made from the main nerve trunk of the middle lateral line after the connections to all but one or a few plaques had been cut.

(i) Spontaneous afferent activity

A high level of spontaneous afferent activity occurs in the lateral line nerves of the tadpole in the absence of applied mechanical stimulation of the end organs. In animals anaesthetized with MS 222 it is greatly reduced but the normal level returns when the effects of the anaesthesia wear off. Spontaneous activity is also reduced after heavy stimulation is applied to the end organs. Thus in Figure 25 (lower record) there is virtually no activity between the periods of stimulation.

(ii) Responses to a vibrating stimulus

The sense organs in a single plaque were stimulated by placing a vibrating tube just above and normal to the skin surface so that it vibrated with an amplitude of about 25 μm in a plane at right angles to the long axis of the plaque (Text-fig. 1). The tubes were made to measure for each preparation and tube diameter was about twice the length of the plaque. Two units could be identified as they were active at different phases of the stimulus cycle (Fig. 25). Displacement of the oscillating stimulation tube from the optimum position (where two units are active) in any direction results in one or both of the units ceasing to fire. The two units can be characterized by their temporal relationship to a given part



Text-figure 1

A tube vibrating in the plane shown was used to stimulate a single plaque. The amplitude of the oscillations was about 25μm.

of the stimulus cycle and by spike height. Presumably each physiological unit corresponds to one of the two morphological classes of receptor cells (see Chapter III) and represents activity in one of the two thick fibres innervating each plaque. No meaningful stimulus-frequency curve was obtained due to the difficulties of recording both units from a single plaque and varying the stimulus frequency without changing the stimulus amplitude.

Groups of organs were stimulated in a similar way using a larger bore tube and higher frequency oscillations. They respond with an initial burst of activity which quickly adapts to a lower level; this is maintained until the stimulus is terminated (Fig. 25).

(iii) Response to varying water current velocities

To test lateral line responses to a current flow, tadpoles were placed in as large a dish as possible (four and a half by six inches) and stimulated with a stream flowing from a narrow bore pipette (tip diameter 0.5 mm approx.) placed close to the part of the lateral line being stimulated (Fig. 27). The strength of the current was regulated by varying the height of a constant head reservoir. The apparatus was first calibrated by measuring the flow rate of methylene blue from the pipette at different elevations of the reservoir. It was recalibrated whenever the pipette was changed although different pipettes had approximately the same physical characteristics. The flow rate was determined using a binocular microscope and measuring the time taken for the dye to travel between two pins, a known

distance apart in the preparation dish. A flow rate of less than 0.1 cm per second could not be distinguished accurately and this was the minimum calibrated current strength.

The pipette was arranged so that the current flowed at right angles to the long axes of a small group of plaques. Spike frequency was measured before, during and after stimulation. For each current strength a total of five records was obtained and the average number of spikes in the first 3.0 seconds of stimulation was calculated. A total of 12 preparations was used and typical results are shown in Figure 26. The organs are extremely sensitive and respond to a current flow as little as 0.1 cm per second. With stronger currents the response increases and the number of spikes is proportional to the logarithm of the current velocity. In all preparations the response starts to level off with a stimulus flow rate of 10 cm per second.

(iv) The directionality of the response to current flow

The plaques of the tail region of the middle lateral line are arranged so that their long axes are roughly parallel (Fig. 3). Therefore it can be assumed that the hair processes in adjacent plaques are orientated in approximately the same direction (see Chapter III) and directionality bestowed upon the organs by morphological features would be the same. The tadpole was arranged so that the central point of the group was at the centre of an arc described by a moveable pipette (Figs 22 and 27). The direction of the stimulating current could be altered without opening the experimental chamber. The height

of the pipette was variable and it was arranged so that the current from it flowed tangentially to the surface of the tadpole. The organs lay in the centre of the flow and so were not affected by eddy currents. A current of 0.5 cm per second was used as previous experiments had shown it to be in the middle of the velocity/response curve (Fig. 26). The pipette was arranged at angles ranging between 0° and 360° with respect to the long axis of the plaque and afferent activity was recorded as above for each position of the stimulus. Spike frequency was measured before, during and after stimulation. A total of five readings for each position was obtained and the average frequency during the first 3.0 seconds of stimulation was calculated. The highest activity was recorded when the current flow was at right angles to the long axes of the plaques and the minimum when the flow was parallel to the long axes.

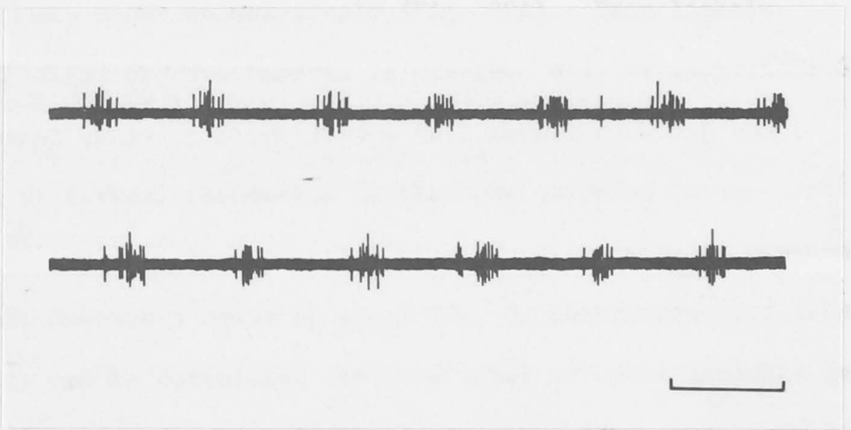
(II) Efferent activity

Efferent activity was recorded from the nerve trunks of the middle and supra-orbital lateral line nerve trunks after all their peripheral connections had been cut. The other lateral line nerves were left intact. No spontaneous activity was found in the lateral line nerves. Heavy MS 222 anaesthesia completely abolishes efferent activity but it returns as the effects of the anaesthetic wear off. However, as the efferent activity returns the tadpole begins to struggle which makes recording almost impossible. For this reason tadpoles were curarized prior to recording but for comparison some records were obtained from animals lightly anaesthetized with MS 222. Apart from movement artifacts which occurred in the lightly

MS 222 treated animals there was no difference between the two types of record. Patterns of activity fall into two classes which are both associated with tadpole movements.

(i) Activity associated with gill movements

During respiratory ventilation of the oral cavity a burst of spikes occurs every time the pharynx floor is lowered. In a normal animal there are about 40 bursts every minute, each lasting about 1/3 second and the pattern of spikes varies from burst to burst (Text-fig. 2). This type of activity can be



Text-figure 2

These records show bursting efferent activity in the middle lateral line nerve trunk.
Scale 1.0 sec.

recorded up to stage 61 when the tadpole mode of respiration is replaced by the adult one. Bursts of activity in the left and right middle lateral lines and in the middle lateral and supra-orbital lines of the same side of the tadpole were in phase. It is interesting that bursting activity persists in tadpoles which have been curarized. This suggests that it is independent of mechanoreceptor input from the gills.

(ii) Activity associated with locomotory movements

Long bursts of efferent activity lasting up to five seconds can be elicited by intense tactile stimulation of the tadpole or they may occur spontaneously (Fig. 30A). From lightly anaesthetized preparations it is apparent that these periods of persistent activity occur during tail movements which would result in forward locomotion in the free swimming larva. At stage 60 the legs become actively involved in swimming movements and tail movements cease by stage 63. In mature tadpoles efferent activity can be correlated with the level of motor activity in the sciatic nerves (Fig. 30B). Again there is a close correlation between levels of activity in different lateral line nerves of the same animal.

(iii) Lack of efferent units responding to lateral line stimulation

All attempts to excite efferent units by stimulation of ipsilateral and contralateral rows were unsuccessful. Two methods were used, a vibrating rod applied to the skin surface and electrical depolarization of the lateral line nerves. Efferent units were intact during these experiments because in many of the preparations

regular bursts of activity associated with gill movements lasted until well after completion of the stimulation (Fig. 30C). Extracellular records of afferent activity in the lateral line rows being stimulated showed that the methods used for stimulation were effective.

Efferent activity could be elicited in silent lateral line nerves by vibrating the whole tadpole with an amplitude of about 0.5 mm at 30 Hz (Fig. 30D). This level of stimulation greatly exceeds the normal physiological range of stimulation the tadpole would experience and could stimulate a variety of mechanoreceptor inputs. In the same way efferent units can be elicited by sharply tapping the preparation dish (Fig. 30E).

SUMMARY AND CONCLUSIONS

Two types of activity, afferent and efferent, occur in the tadpole lateral line nerves. Each plaque is innervated by two afferent units which are spontaneously active, sensitive to mechanical displacement of the hair processes and respond in a phasic/tonic manner to high frequency vibrations. Lateral line plaques are sensitive to water currents and the response is proportional to the logarithm of the current speed. They respond maximally when the current flow is at right angles to the long axes of the plaques. This directional response is consistent with the morphological polarization of the hair processes (see Chapter III).

Efferent units are not spontaneously active but fire during gill and tail movements. Levels of activity are comparable in different lines of the same animal. Ipsilateral and contralateral

stimuli fail to excite efferent units in the tadpole although similar stimuli are known to excite efferent units in the adult (Görner, 1967).

It is concluded that the larval system is functional by stage 54, the plaques are directionally sensitive and they respond to stimulation in the same way as the adult organs (Görner, 1961, 1963; Harris and Milne, 1966). However, the patterns of efferent activity are different in larval and adult stages and this finding is considered more fully in the discussion section.

Figure 22

This diagram shows the arrangement of the recording chamber. The tadpole was anaesthetized with MS 222 and pinned to a wax bottomed dish with enough saline to cover the animal. The preparation dish was covered with two sheets of thin perspex, the nearer one being removable to allow access to the preparation. The electrode holder (e) was introduced through a hole in the perspex cover and the vibrating rod or tube (r) could be introduced through another aperture. A pipette was mounted on a holder (h) and could be turned through an arc of 180° about a central point. Wet blotting paper in the outer chamber kept the humidity high enough to prevent the nerves from drying out.

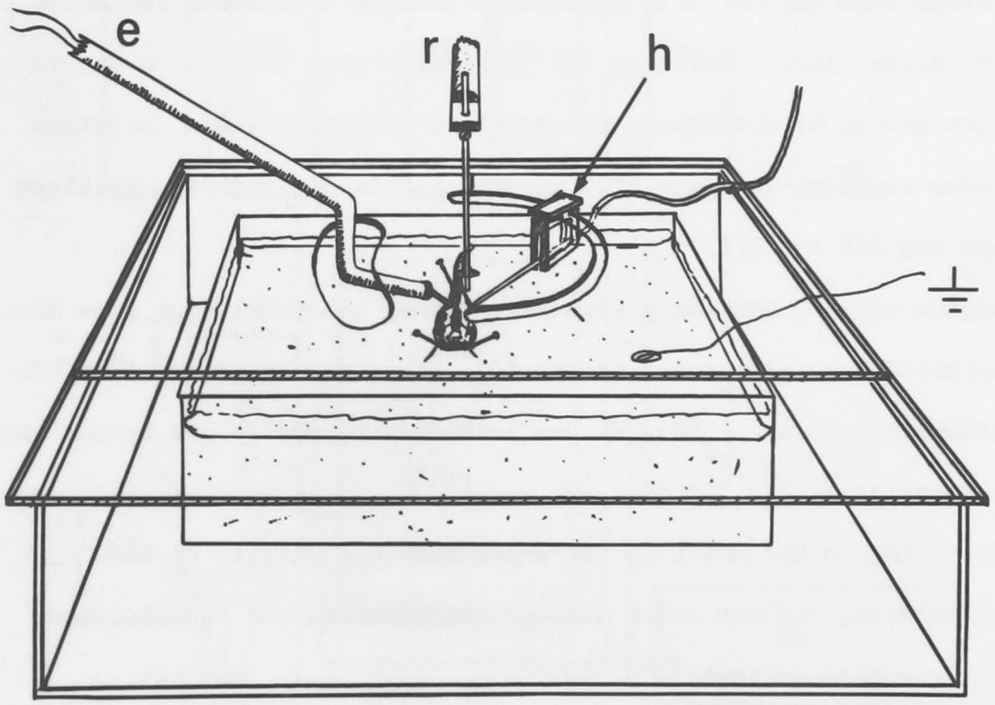

e
r
h

Figure 23

The circuit diagram summarizes details of the recording and stimulating arrangement. Nervous activity was recorded differentially using Tetronix 122 low level preamplifiers (PA). Amplifier output was displayed on a Tetronix 502 oscilloscope (CRO) and simultaneously recorded on tape using a Ferrograph Series Seven recorder (TR). Nerves were electrically stimulated through a stimulus isolation unit (S) driven from a Tetronix 161 pulse generator (PG) which was in turn driven by a Tetronix 162 waveform generator (WG). Separate power supplies (PS1 and PS2) were available for the preamplifiers and the 161 and 162.

Organs were mechanically stimulated using an oscillating tube mounted on an Advance vibrator type six (O). This was driven from an Advance sinewave generator (AG), it was necessary to modify its output using a transformer (T).

According to the needs of the experiment the outputs of the stimulating devices could also be displayed on the oscilloscope and recorded on tape.

To record the commencement of water current flow from a fine pipette a switch was attached to the tap controlling the flow. When the tap was opened an electrical circuit was completed and a DC displacement recorded on the oscilloscope screen. Similarly when drops of water falling onto the water surface were used to stimulate the lateral line system each drop completed a circuit as it hit the water surface.

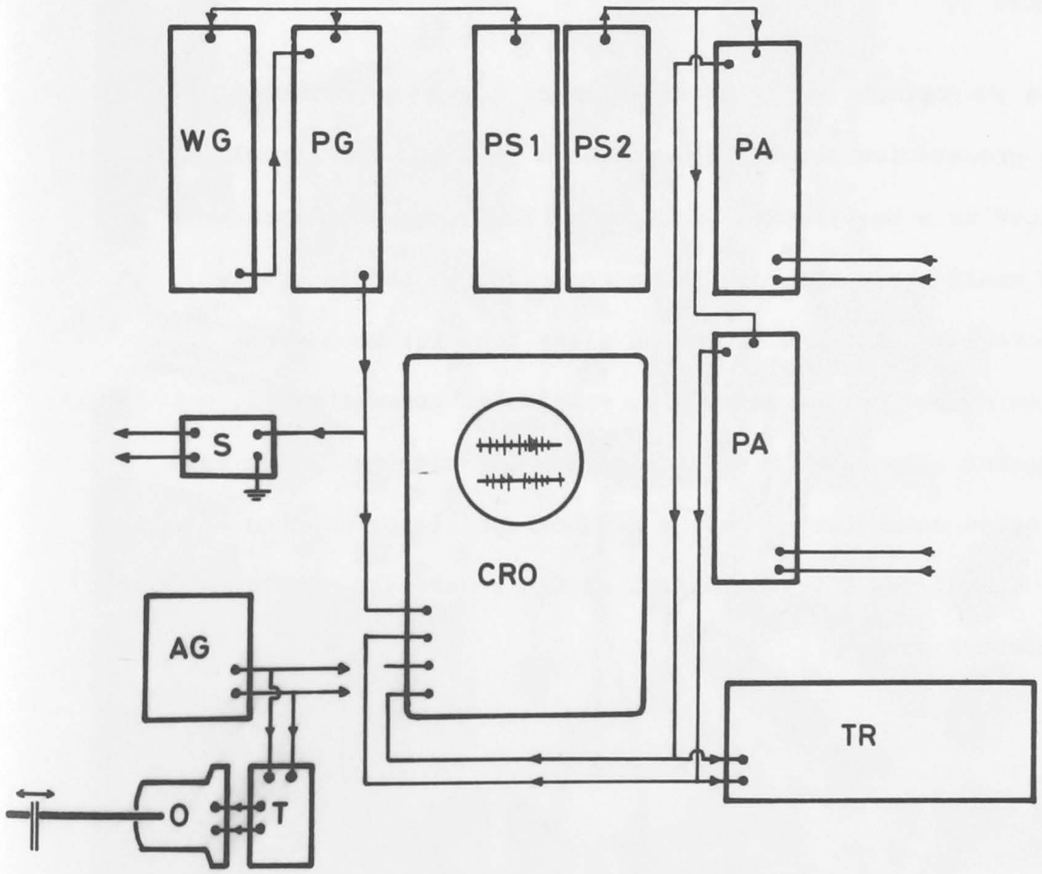

WG
PG
PS1
PS2
PA
PA
S
CRO
AG
TR
O
T

Figure 24

This photograph shows the arrangement of the experimental set-up. The preparation chamber (p) stood on four one inch steel legs bolted to a heavy steel baseplate. The cover of the chamber had small apertures for the introduction of the recording electrodes (e) and a vibrating glass tube for mechanical stimulation. c. = camera; g. = stimulus generator; l. = lamp for illumination of specimen; m. = microphone for tape recorded commentary; osc. = Advance oscillator mounted on a lathe bed; s. = physiological saline reservoir; st. = stimulus isolation unit.

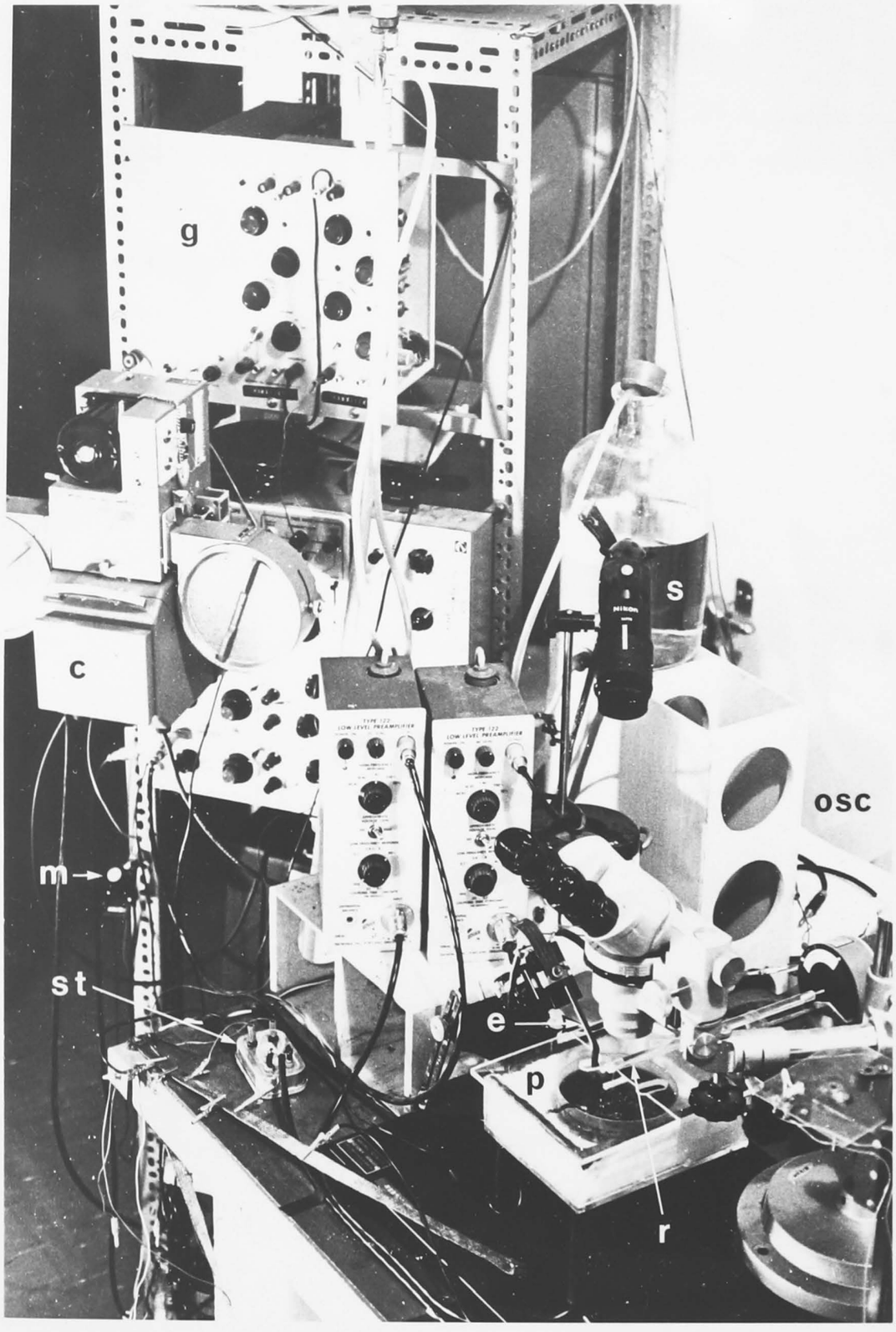

g
s
c
osc
m
st
e
p
r

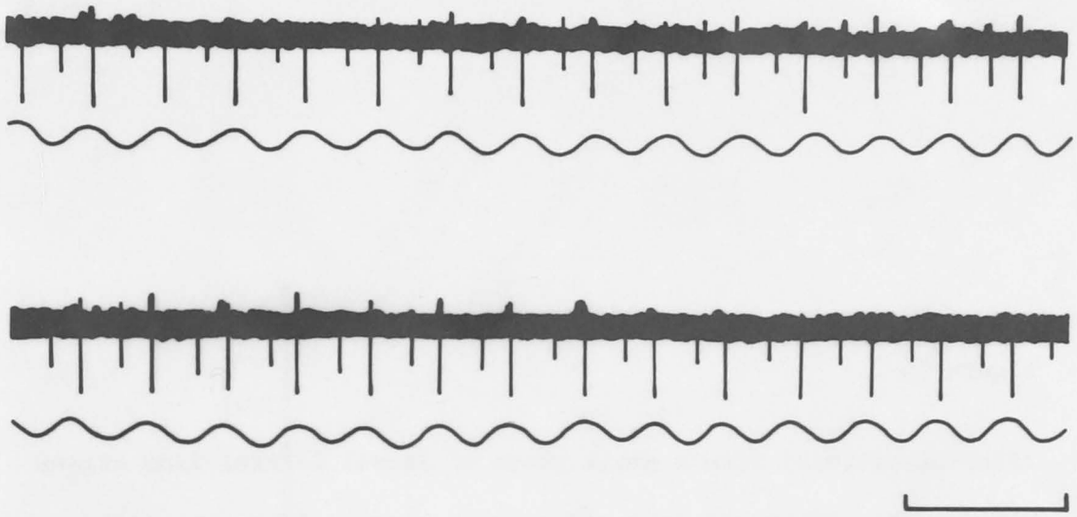
Figure 25

This record shows sensory two units from a single larval plaque responding to sine wave vibrations of a tube placed over the top of the organ plaque and oscillated in a plane at right angles to its long axis. The two units fire at different parts of the stimulus cycle; by advancing or retracting the vibrating tube parallel to the animal's surface and at right angles to the organ's long axis, it was possible to eliminate one or other of the units.

Scale 0.25 seconds.

The illustration opposite shows the response of a group of organ plaques to a higher frequency oscillation. Record A shows that an initial high level of activity is followed by a lower tonic level. Records B, C and D are consecutive parts of the same trace; they illustrate the phasic and tonic parts of the response and show that the tonic activity persists over a long period of time. The level of spontaneous activity between stimuli is low.

Scale 1.0 seconds.



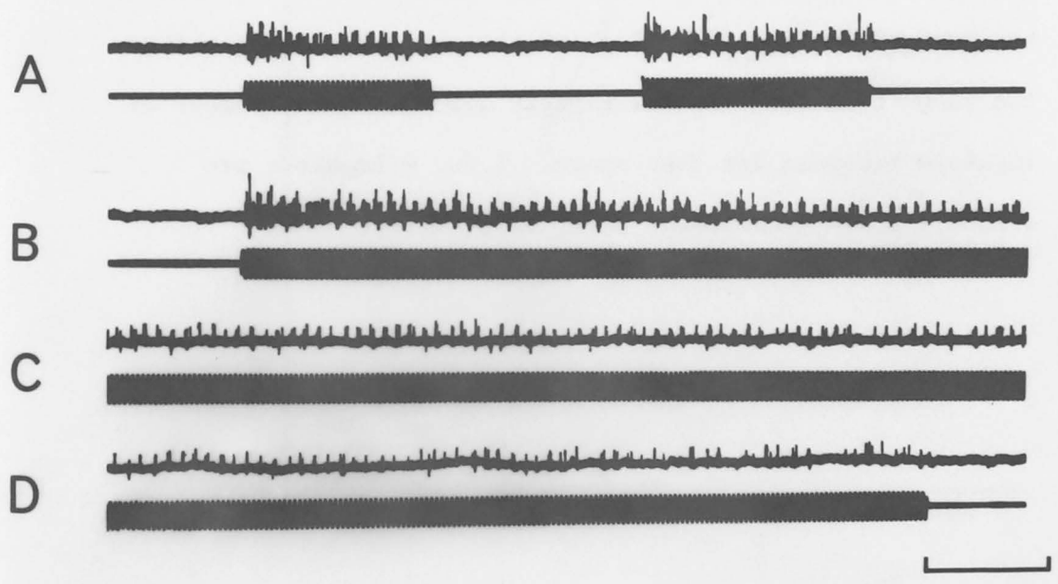

A
B
C
D

Figure 26

Afferent activity from a small group of larval lateral line organs showing the effects of increasing water current flow upon spike frequency. The organs were from the middle lateral line and had approximately parallel long-axes. There were two or three plaques in the group and the activity recorded represents their combined outputs. There is a clear relationship between current flow and the number of impulses per second. Each point on the graph is the average of five recordings at the same current velocity; the range bars indicate the highest and the lowest number of impulses recorded for that speed. i./s. = impulses per second; sp. = spontaneous level of activity.

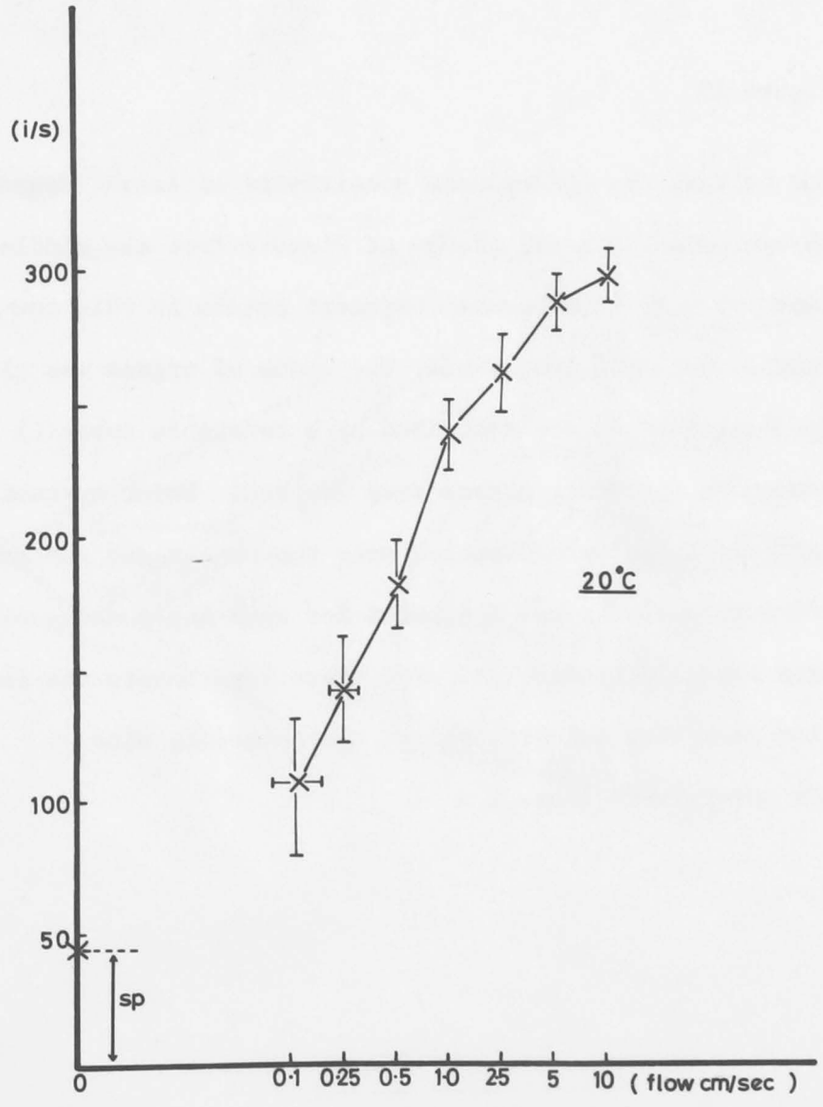

(i/s)
300
200
100
50
sp
0
0·1
0·25
0·5
1·0
2·5
5
10
(flow cm/sec)
20°C

Figure 27

For testing the directional sensitivity of larval organs physiologically small groups of plaques from the middle lateral line (m) were used because adjacent organs in this row have roughly parallel long axes. The group of organs was placed at the centre of an arc described by a rotatable tube (t) and the nerves to all other organs were severed. Water currents from various angles were directed onto the organs and the level of afferent activity was monitored for each angle using silver wire hook electrodes (e). For these experiments the lateral line nerve was cut proximal to the recording site.

d = upper later line.

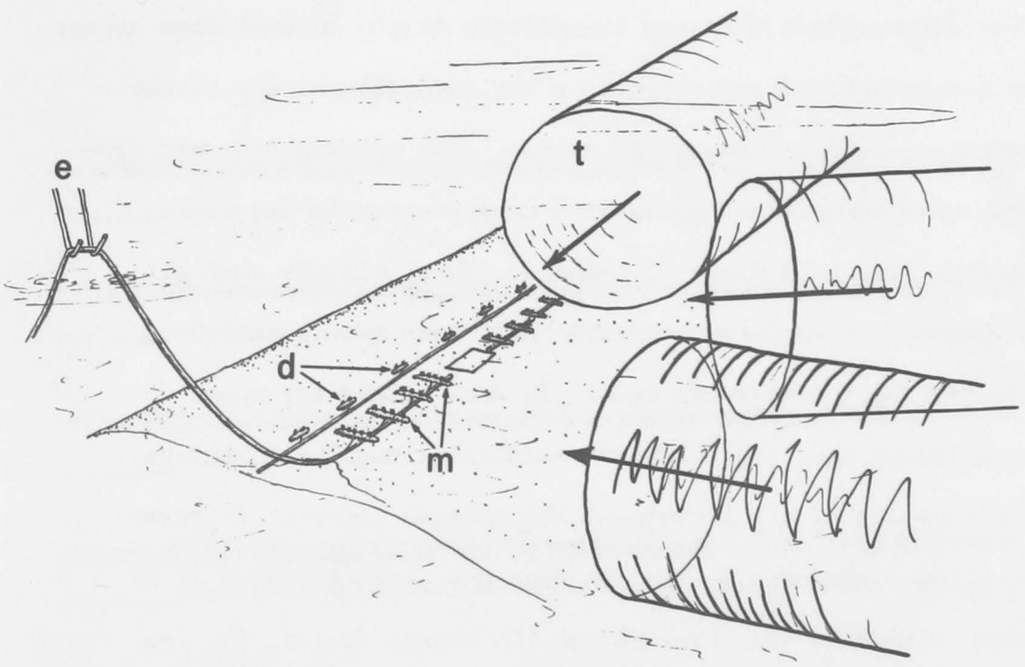

e
t
d
m

Figure 28

This figure shows that the sensitivity of the lateral line organs to a constant current changes as the direction of the current source is rotated around the organs. The records A-C were from the nerves of a small group of 3 or 4 plaques in the middle lateral line of a stage 55 tadpole. These plaques were all orientated in the same way with their long axes parallel to the tadpole's transverse axis. In each record the upper trace shows the level of nervous activity and the lower one the duration of the stimulus. The maximum response in terms of spike frequency is obtained when the current source is at right angles to the long axes of the organs (record E). As the source of the current is moved from this position (90°) in an arc around the organs the response to the stimulus decreases (records D, C, B, A). The minimum response is recorded when the current source is in the plane of the long axes of the organ plaques (record A) at 0°. If the current source is rotated further, a second maximum is recorded at 270° (not shown here). Scale 1 sec.

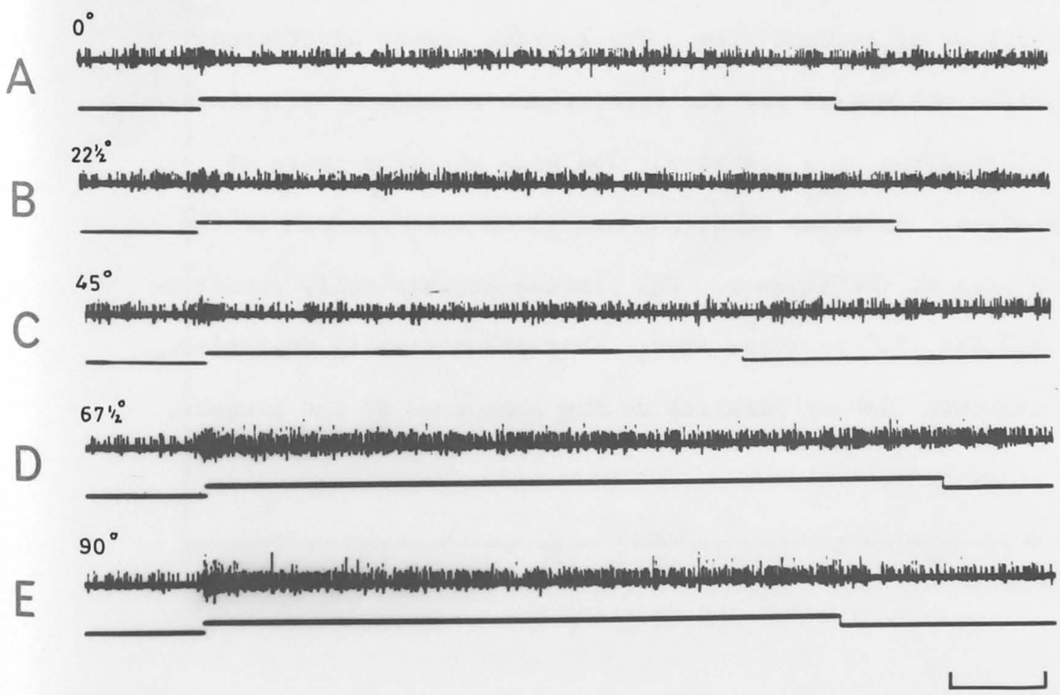

0°
A
22½°
B
45°
C
67½°
D
90°
E

Figure 29

The directional sensitivity of two small groups of plaques is shown here. Five recordings were made for each of nine directions of current flow. The average number of afferent impulses per second for the first three seconds of stimulation is plotted for each position. The bars show the range of variation. Stimulus positions are given with respect to the long axes of the plaques. The plaques are maximally sensitive at 90° and 180° to these axes. They are minimally sensitive to currents flowing parallel to the long axes of the plaques.

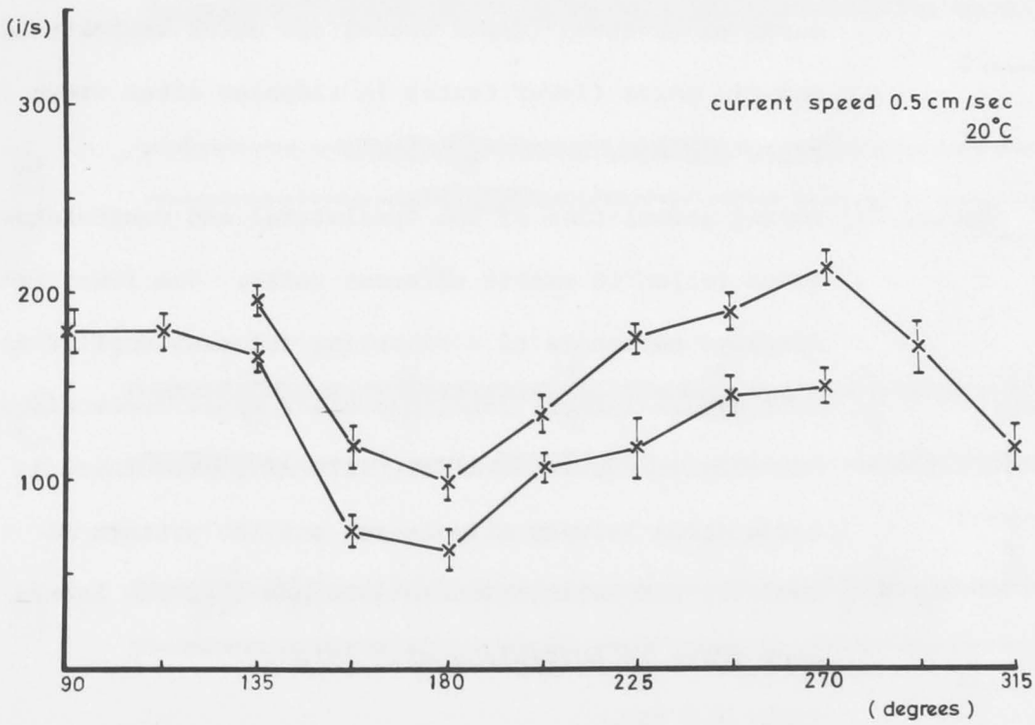

(i/s)
300
200
100
current speed 0.5 cm/sec
20°C
90
135
180
225
270
315
(degrees)

Figure 30

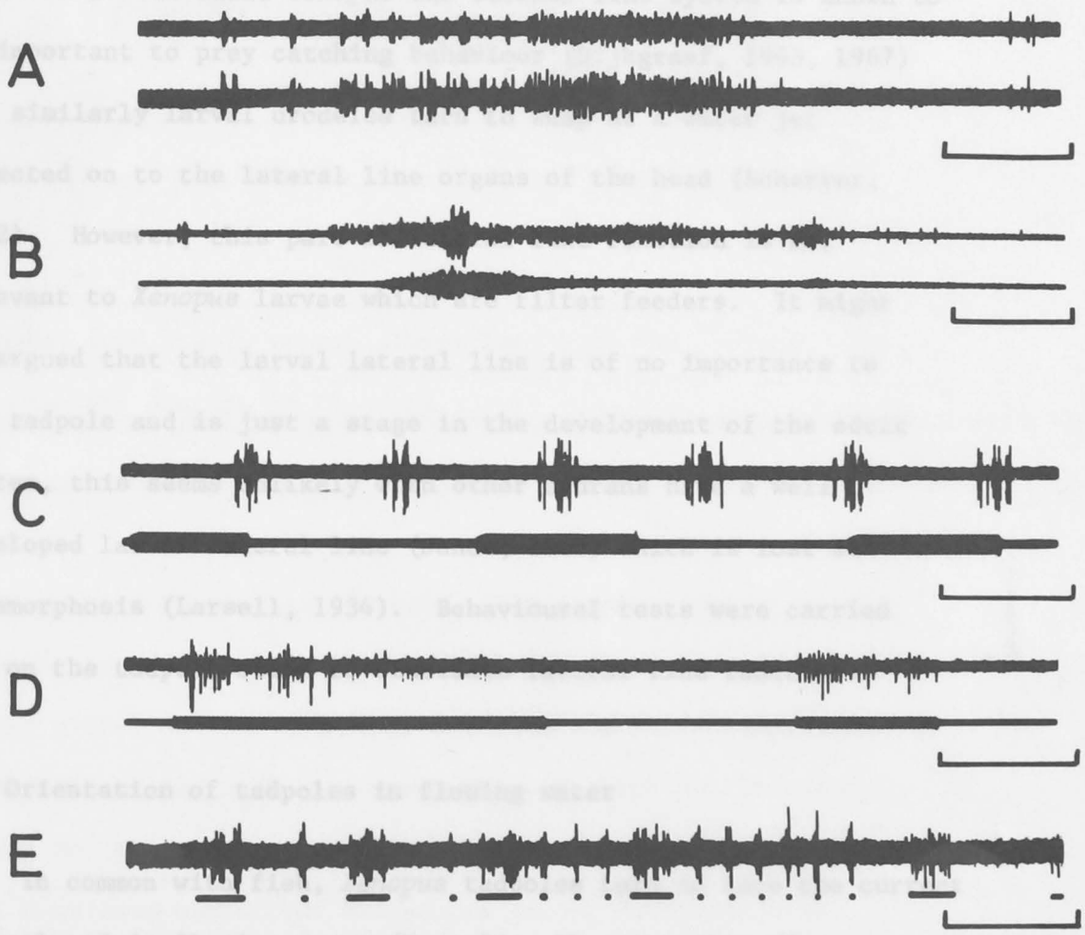
Record A. Long periods of efferent activity occur during tadpole movements. The pattern and level of activity is similar for the middle lateral line nerves on the left (upper trace) and right (lower trace) of the animal.
Scale 1.0 sec.

Record B. There is a strong correlation between efferent activity in the lateral line nerves (right middle lateral line nerve shown here) (upper trace) and motor activity in the sciatic nerve (lower trace) in tadpoles after stage 60.
Scale 0.5 sec.

Record C. Strong stimulation of the ipsilateral and contralateral lines failed to excite efferent units. The lower trace displays movements of a vibrating stimulus applied to the left middle lateral line, the upper trace shows efferent activity in the right middle lateral line. There is no correlation between stimulation and the pattern of activity but bursting units indicate that the lateral line nerve is physiologically intact.
Scale 1.0 sec.

Record D. Strong vibrations with an amplitude of 0.5 mm applied to the whole tadpole (lower trace) excite efferent activity in the middle lateral line nerve (upper trace) but such strong stimulation would not be encountered in normal life.
Scale 1.0 sec.

Record E. Similarly, sharp taps on the preparation chamber (dots) excite efferent activity between the more regular bursting efferents (marked by bars).
Scale 1.0 sec.


A
B
C
D
E

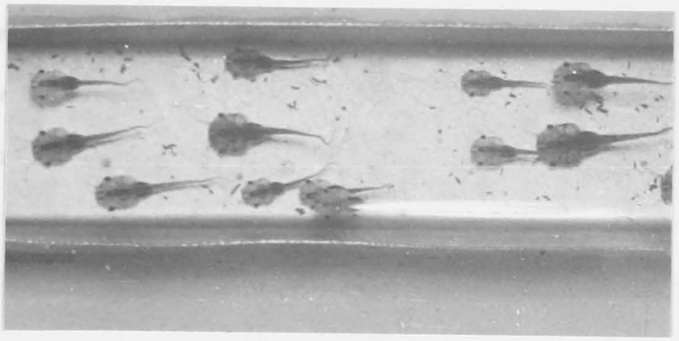
CHAPTER V

THE LATERAL LINE IN RELATION TO THE BEHAVIOUR OF *XENOPUS* TADPOLES

The role of the lateral line in the behaviour of anuran tadpoles is unknown. In adult *Xenopus* the lateral line system is known to be important to prey catching behaviour (Dijkgraaf, 1963, 1967) and similarly larval urodeles turn to snap at a water jet directed on to the lateral line organs of the head (Scharrer, 1932). However, this part of lateral line function is not relevant to *Xenopus* larvae which are filter feeders. It might be argued that the larval lateral line is of no importance to the tadpole and is just a stage in the development of the adult system, this seems unlikely when other anurans have a well developed larval lateral line (Jande, 1966) which is lost at metamorphosis (Larsell, 1934). Behavioural tests were carried out on the tadpole stage to elucidate lateral line function.

I. Orientation of tadpoles in flowing water

In common with fish, *Xenopus* tadpoles turn to face the current when placed in flowing water (Text-fig. 3). A number of experiments were devised to test the role of the lateral line system in this response. The lateral line nerves on the left side were cut at the central end as near to the brain case as possible in 20 stage 54 - 56 animals. A second group of tadpoles had their nerves cut on both sides. The tadpoles were allowed one day to recover from the operation and each batch was placed in a



Text-figure 3

Tadpoles in a steady stream always face into the current. They can maintain station in the dark.

---

flowing water chamber in the dark. The chamber consisted of a metal channel two feet long, of U-shaped cross section, two inches wides, one and a half inches deep and containing water to a depth of one inch. A steady flow of water was introduced at one end and overflowed via a spillway at the other. This gave a uniform current of 0.5 cm per second in the central one foot section which contained the tadpoles. Fine gauze barriers separated this central region from the ends. Care was taken to eliminate temperature gradients by placing the channel in a constant temperature bath at 18°C. Still photographs of experimental larvae and normal controls were taken at intervals of half-hour over periods of three hours

using a still camera and an electronic flash. These photographs showed that the operated tadpoles faced the current in the same way as the controls. In addition, both operated and control tadpoles were usually evenly distributed along the length of the chamber which indicates an ability to maintain station.

II. The turning response

Xenopus tadpoles normally remain stationary in mid-water or move very slowly across a tank of still water. In this quiescent state a turning response can be elicited which is similar to the one described for urodele tadpoles (Scharrer, 1932). The tadpole turns towards the stimulus with a rapid tail flick but does not swim towards it. A fine jet of water directed at the head region, approaching surface waves spreading from a drop hitting the water and a vibrating rod placed at a distance on one side of the tadpole are all effective stimuli (Fig. 31). The response is obtained in as few as 15 per cent of the trials. Similar behaviour is known in fishes (Schwartz, 1967) but the stimulus always evokes a response and the fish swims towards the stimulus.

To determine the accuracy of the orientation of tadpoles to disturbances, a large number of trials were carried out on stage 54 - 56 animals using drops hitting the water surface as a stimulus. The tadpoles were placed in a 12 x 15 inch dish with water depth of one inch. To place the drops accurately with respect to the tadpole, four holes were drilled in a protractor on the 30°, 60°, 90° and 120° radii five centimetres from the baseline (0° - 180°). The tip of a Pasteur pipette was

pushed into one of the holes until it was held firmly. Using the pipette as a vertical handle the protractor was held above and parallel to the water surface over the tadpole. The baseline was aligned to the antero-posterior axis of the tadpole with the centre of the baseline above the medulla of the brain. Single drops were pipetted on to the water surface from a height of one centimetre and the reorientated position of the tadpole was read directly from the protractor. In normal animals, 18 responses were obtained from 102 trials; the reorientation was most accurate when the waves approached from the front, and less accurate to waves from behind (Fig. 32). Two control animals with severed optic tracts did turn towards the stimulus which indicates the response is not a visual one. Tadpoles with all lateral line nerves cut did not respond.

In adult *Xenopus* similar stimuli elicit a much more complex response; the toad turns towards the stimulus, approaches it and attempts to engulf the source (Fig. 33). The response is more readily obtainable especially in unfed toads which nearly always respond. Actively swimming larvae and adults do not make the response.

III. Tadpole behaviour in which lateral line function may be involved

Unrestrained, free-swimming tadpoles observed in a tank displayed several behaviour patterns which involve the orientation of larvae with respect to one another and which are probably important to feeding behaviour (Fig. 34). Commonly two tadpoles lie more or less stationary above the substrate

with their bodies parallel and separated by a distance of one centimetre or so (Fig. 34A). They periodically ventilate the oral cavity and are clearly filter feeding. By adopting an aligned position neither tadpole refilters water already depleted of food by the other one. Tadpoles will also swim slowly across the tank in pairs on parallel courses (Fig. 34B). The function of this behaviour may be similar to the first type. In both cases the lateral line system may be used to maintain the parallel orientation. More than two stationary tadpoles can be involved in similar patterns of behaviour. The result is the formation of a loose shoal in which the tadpoles all face the same way (Fig. 34C). The shoaling behaviour also increases filter feeding efficiency. Avoidance responses were also observed, one tadpole changing its direction of swimming to pass another stationary or slowly swimming tadpole on an opposite but parallel course (Fig. 34D). Once again the lateral line system may be used to detect water disturbances caused by another tadpole.

SUMMARY AND CONCLUSIONS

Although the lateral line system is not essential for the orientation of tadpoles in flowing water, the turning response shows the ability to detect differential stimulation of the system. This response is not elicited as readily in tadpoles as it is in fishes and its accuracy depends upon the direction of the stimulus. A stimulus in front of the tadpole elicits a more accurate response than one arising behind it. It is worth mentioning that inaccuracies of the turning response may not be due solely to the lateral line system but could also reflect

an inability of the motor system to turn the tadpole through large angles with a single tail flick. The importance of the adult lateral line in feeding behaviour is confirmed. Several patterns of tadpole behaviour are described which could involve the lateral line system.

Figure 31

This series of ten photographs taken from a ciné film shows a tadpole responding to a jet of water from a pipette on the left of the animal. The tadpole completes its turn towards the pipette in 1/2 second, the consecutive pictures being separated by 1/16th of a second. The tip of the pipette has been painted black in this instance to make its position clear.

1

2

3

4

5

6

7

8

9

10

Figure 32

The accuracy of the turning response of a tadpole at stage 56 is shown diagrammatically. The stimulus was a water drop falling from a height of one centimetre on to the water surface at a distance of five centimetres from the tadpole. The initial orientation of the tadpole is indicated by the heavy arrow. The point where the drop hit the water is shown by the arrow-head and each diagram plots the final orientation to stimuli at a fixed angle to the antero-posterior axis of the animal, indicated by light arrows. Tadpoles respond most accurately to waves approaching from the front. Stimuli from behind the animal result in less accurate responses with insufficient turning to face the direction of stimulation.

60°
120°
30°
90°

Figure 33

This ciné sequence shows the typical adult response to a fine water jet. The toad turns towards the source and starts feeding behaviour. It sweeps the area in front of the mouth with the forelimbs and swims after the pipette if it is moved away. The pictures in the series cover a period of $2\frac{1}{2}$ seconds and are selected from a film exposed at 32 frames per second.

1

2

3

4

5

6

7

8

9

10

Figure 34

These four photographs of stage 55 tadpoles in still water show various common behaviour patterns which may involve lateral line function.

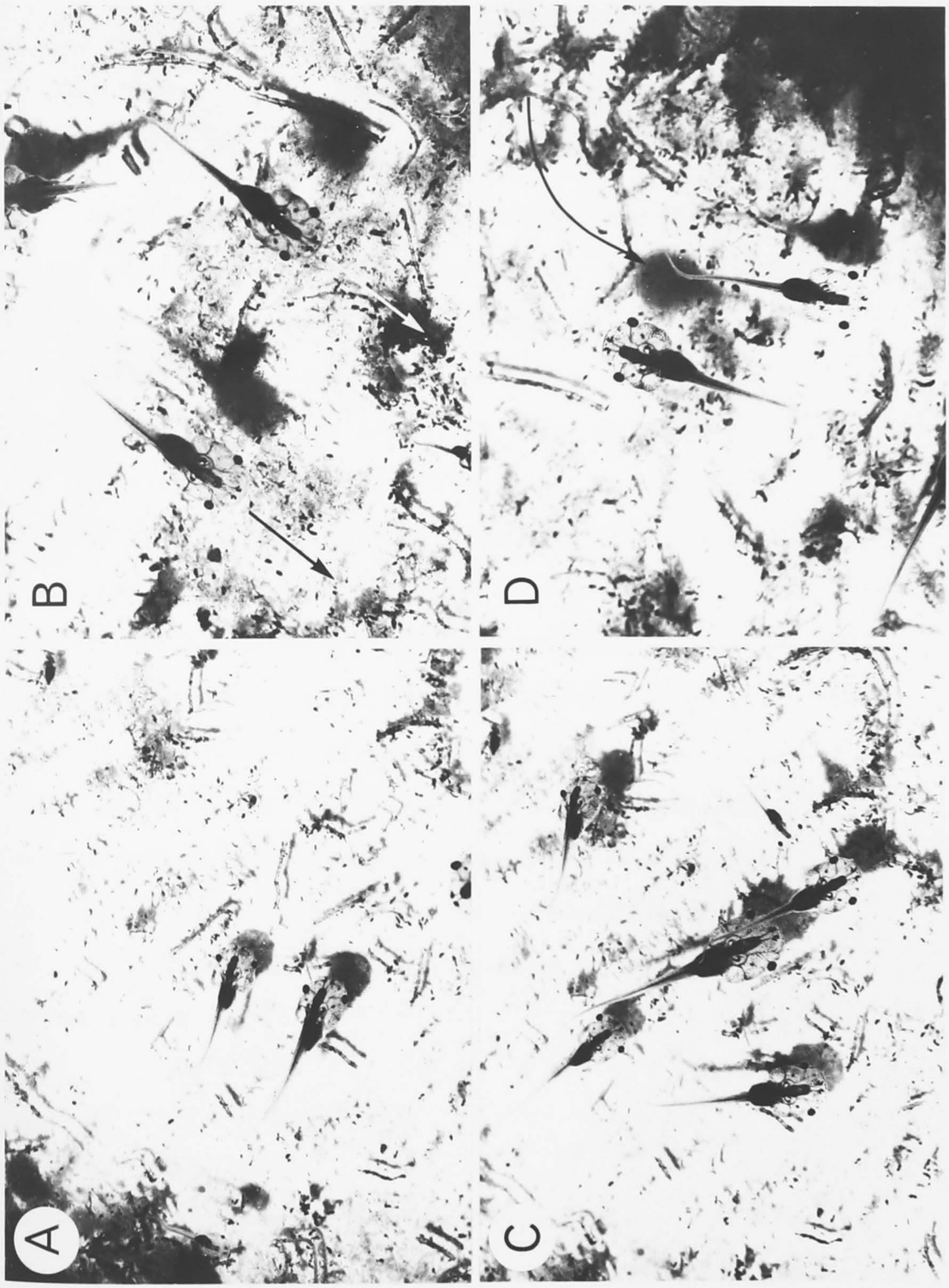
(A) Two tadpoles in a "stationary" feeding position, the distal region of their tails continually undulating. They are aligned parallel to one another.

(B) This pair of larvae are swimming across the tank parallel to one another at the same velocity.

(C) Shoaling behaviour is exhibited by small groups of animals. The shoal formation can involve as many as a dozen animals at one time. As one animal leaves the shoal another may join it.

(D) This photograph shows avoidance behaviour, the left hand tadpole is stationary and the other one has approached from the right (arrow). The two animals pass on parallel but opposite courses.

All of these manoeuvres probably involve the visual and other sensory systems but the lateral line could be useful in assessing distance.


A
B
C
D

CHAPTER VI

METAMORPHOSIS OF THE LATERAL LINE SYSTEM IN *XENOPUS*

During metamorphosis the supra-orbital and post-orbital lines migrate to a new position around the orbit of the eye (Paterson, 1939). This movement involves changes in the orientation and location of plaques so that at different developmental stages the same plaque faces different sectors of the environment. Since the directional sensitivity of plaques is established before this redistribution takes place (see Chapter IV) the implication is that the central nervous system must compensate for the peripheral changes. The present account shows that similar reorganization occurs in other lines and discusses the role of the supra-orbital line before and after metamorphosis. Developmental changes in gross morphology of the plaques are reported, the maturation of myelinated lateral line axons is described and the conduction velocities of lateral line nerve fibres are examined before and after metamorphosis.

RESULTS

I. Changes in position, number and orientation of the lateral line organs at metamorphosis

The distribution of lateral line rows in larva and adult is shown in Figures 3 and 35 respectively. The names of the

subdivisions of the rows are listed in Table 1 together with data regarding the position of the rows, the number of plaques found in each row and the angles at which plaques are orientated with respect to the antero-posterior median axis. These data were collected by the examination of whole animals and from whole mount skin preparations of five stage 55 tadpoles and five stage 66 newly metamorphosed adults. Intermediate stages were examined to relate the larval and adult patterns of organ distribution.

The number of plaques in many of the rows is approximately the same in larva and adult although in some there is a drastic reduction in numbers. The loss takes place because some areas of larval skin are not replaced by the adult skin and the plaques in these regions disappear at metamorphosis (Nieuwkoop and Faber, 1967). The anterior auditory groups, the aortic row and the tail portions of the middle, lateral and upper lateral lines are lost completely. Numbers of plaques in the hyomandibular, anterior lower lateral and caudal rows are severely reduced (see Table 1). In certain rows there appears to be an increase in plaque number at metamorphosis, which is probably due to confusion in knowing where one row begins and another ends and does not represent formation of new organs. There is, for instance, a large reduction in the number of plaques in the anterior lower lateral line and an increase in the posterior lower lateral lines.

Lines which are dorsal, lateral or ventral in the larva often maintain these positions in the adult; however, the infra-orbital, tentacular, anterior lower lateral, caudal and

Table 1

Table 1 opposite shows the differences in distribution of lateral line rows in larvae and adults. Information was gathered from an examination of five tadpoles and five adults. The position of each row and the numbers of plaques in them are recorded. The orientation of plaques was determined by extrapolating lines through their long axes and measuring the angles subtended with the median antero-posterior axis. Reference axes for the measurement of these angles are shown in Figs 3 and 35.

(From Shelton, 1970.)

Table 1. *Changes in the lateral line rows at metamorphosis*

Lateral line row	Stage 55 larvae			Stage 66 adults		
	Position of row	Number of plaques in row	Angle to reference axis	Position of row	Number of plaques in row	Angle to reference axis
Supra-orbital complex						
(*a*) Parietal	Dorsal	3–4	150–180	Dorsal	3–5	120–140
(*b*) Supra-orbital	Dorsal	10–15	70–90	Dorsal	12	0–170
Infra-orbital complex						
(*a*) Post-orbital	Dorsal	4–6	60–70	Dorsal	3–4	130–170
(*b*) Infra-orbital	Lateral and ventral	5–6	90–160	Dorsal	4–5	10–40
Hyomandibular complex						
(*a*) Tentacular	Ventral	3–5	15–45	Lateral	4	10–70
(*b*) Pre-orbital	Ventral	3–6	60–140	Ventral	4–5	50–140
(*c*) Mandibular	Ventral	9–12	100–160	Ventral	8–9	140–160
(*d*) Hyomandibular	Ventral	9–15	30–120	Ventral	3–4	140–160
Anterior auditory	Dorsal	5–8	70–120	Absent	– –	– –
Occipital	Dorsal	7–10	20–150	Dorsal	8–10	150–170
Aortic	Dorsal, lateral and ventral	7–9	–10–30	Absent	– –	– –
Lower lateral complex						
(*a*) Anterior l.lat.	Lateral and ventral	12–18	10–120	Ventral	4–5	0–30
(*b*) Posterior l.lat.	Lateral and ventral	8–12	70–170	Ventral	20–22	30–140
(*c*) Median ventral	Lateral	3–4	80–90	Ventral	3–5	160–180
(*d*) Caudal or anal	Lateral	15–20	15–20	Ventral	4–5	0–10
Middle lateral						
(*a*) Trunk region	Dorsal and lateral	25–30	60–120	Dorsal and lateral	26–34	50–120
(*b*) Tail region	Dorsal	Many	80–90	Absent	– –	– –
Upper lateral						
(*a*) Trunk region	Dorsal and lateral	18–22	20–150	Dorsal and lateral	16–24	0–140
(*b*) Tail region	Dorsal	Many	170–180	Absent	– –	– –
Posterior auditory	Dorsal	7–9	70–130	Lateral	5–7	100–110

posterior auditory rows show distinct changes of position at metamorphosis (see Table 1).

The orientation of the long axes of plaques with respect to the median antero-posterior axis of the animal were measured in larvae and adults. For each row the range of angles to the reference axis was ascertained and the extremes noted.

Large pieces of skin containing left and right rows were removed and photographed. The median antero-posterior axis was marked on the photograph and the angles of plaques to this axis were measured. There is a curvature of the animal's surface and in making the final assessment of angles, reference was made to whole mount preparations. Using this technique the angles could be assessed to an accuracy of ±5°. While plaques in certain lines maintain approximately their larval orientation, many do not. These include the post-orbital, hyomandibular, occipital, anterior lower lateral, median ventral and caudal lines (see Table 1).

In the anterior lower lateral and caudal lines there are changes in position, number and orientation of organs. In the hyomandibular and posterior lower lateral lines there are changes in two of these parameters. In all lines there is some change in at least one of the three features examined. The mechanisms resulting in the changes were not examined but the reorganization during development probably reflects changes in animal shape and factors such as uneven stretching of the skin. A functional explanation for the changes in one particular line is detailed below.

II. The supra-orbital line at metamorphosis

(i) Changes in plaque distribution

Apart from the total loss of some lines at metamorphosis, the most striking change in plaque distribution occurs in the supra-orbital row. Prior to metamorphosis, the plaques are arranged one behind the other with their long axes roughly parallel and approximately perpendicular to the antero-posterior axis of the tadpole (Fig. 3). Thus most of the plaques face the same sector of the environment. A water displacement occurring in this sector would stimulate all organs more or less equally (Fig. 36). Consequently the ability of the supra-orbital system to resolve a stimulus within this sector would be little more than the limit imposed by the characteristics of the bell shaped stimulus/response curve of a single sensory unit (Görner, 1963) because there is little angular separation of peak sensitivities of adjacent plaques.

In the adult, the plaques of the supra-orbital line are arranged radially around the eye (Fig. 35) and each one faces a slightly different part of the environment (Fig. 36). This arrangement makes the supra-orbital line sensitive to disturbances in a wider sector and increases the accuracy with which a stimulus within that sector could be localized. The key factor in these improvements is that angular separation of the peak sensitivities of adjacent receptors is sufficient for a single stimulus to fall in different regions of the sensitivity curves of adjacent receptors. Similar receptor arrays for

providing accurate localization of a stimulus are known in a number of other sensory systems such as fish otolith organs (Lowenstein and Roberts, 1950) and crustacean statocysts (Cohen, 1955, 1964). As in other sensory systems composed of parallel receptor inputs with divergent angular sensitivities, analysis at a central level capable of providing accurate spatial localization requires integration between adjacent and subadjacent inputs.

The further significance of the rearrangement of the supra-orbital system is discussed in the following sections.

(ii) Surface waves and the supra-orbital system

Adult *Xenopus* come to the surface to breathe and in this position cannot entirely rely on the visual system to detect predators or prey because the environment below the water surface is outside the visual field (Fig. 37). Consequently the lateral line system is especially important at this time. When the toad is in the breathing position the orbital and parietal organs are just below the water surface. The location of organs on the dorsal part of the head is favourable for the detection of disturbances at the surface. Because the amplitude of a surface wave decreases rapidly with depth (see Appendix II) organs on other parts of the body would be less sensitive to surface phenomena. In this context it may be significant that surface-feeding fish respond to surface waves only when they are immediately below the surface (Schwartz, 1970).

(iia) Response of supra-orbital units to natural stimuli

The sensitivity of units to surface waves caused by natural stimuli was investigated by recording afferent activity from the right supra-orbital nerve while an insect struggled on the surface. An adult toad was anaesthetized (see Chapter II), pithed and allowed to recover from the effects of the anaesthetic. The toad was mounted in a 10 x 12 inch dish containing saline (see Appendix I) to a depth of one inch with the eyes and snout just breaking the surface. The nerve trunk to the supra-orbital plaques was exposed, cut centrally to the recording site and divided into small bundles of fibres. Individual twigs containing a few fibres were lifted above the water surface and placed upon silver wire hook electrodes. A second instar locust was floated on the water surface 10 cm from the right eye. The level of afferent activity in the nerve increased in response to very small movements of the locust (Fig. 38).

(iib) Directional sensitivity of units to surface waves

The directional sensitivity of units to artificially produced surface waves was tested using four similar preparations. Drops falling from a height of 1 cm were placed at various points on a circle of 10 cm diameter around the eye. By recording from small bundles of fibres, six single units were identified which responded to surface waves approaching the eye from a restricted angle. All of these had fields no greater than 45° wide (Fig. 38).

(iii) Interpretation of the results

Prior to metamorphosis little time is spent close to the surface, therefore the supra-orbital system of the tadpole is not primarily concerned with the detection of surface waves. Alone the larval supra-orbital line is poorly adapted for the precise localization of stimuli but combined with the inputs from other lines localization could be quite accurate. In the adult the supra-orbital system is probably important in the detection of surface waves. Since small amplitude waves will only stimulate organs just under the surface, stimulus localization could rely less upon comparison between the supra-orbital and deeper lines. Redistribution of the organs of the supra-orbital line increases the angular separation of their peak sensitivities and improves the functional performance of the individual row. This suits it for its specialized role in the detection of surface waves.

III. The surface structure of larval and adult organs

At metamorphosis the skin thickens, the distance between the basement membrane and the surface increasing from 30 μm in the larva to 100 μm in the adult. The lateral line organs in the larva are about 50 μm deep and in consequence their outer surface protrudes above the level of the surrounding skin

(Figs 5A and 39). The adult organs increase in size to 80 μm deep but sink down into the skin. At the same time tactile organs develop between the organs in each plaque (Figs 39 and 40) clearly separating neighbouring organs. There is no evidence that these changes affect lateral line function although the tactile receptors may shield the organs from currents parallel to the long axis of a plaque. This could increase the directional response.

IV. Structural changes in the innervation of single plaques at metamorphosis

Examination of several bundles innervating single organ plaques in the larva revealed two morpholgoically distinct groups of fibres; two myelinated fibres approximately two - four μm in diameter (Chapter III). This finding is in contrast to the situation described for the adult where an additional class of smaller thin myelinated fibre is often present. Because some plaques in the adult lack an innervation by thin fibres (Murray, 1955) it is not possible to confirm its absence in the tadpole from a small sample. For this reason a large number of tadpole plaque innervations were examined to confirm the absence of the small myelinated fibre class and to determine the stage in development at which it appears.

Results of this study are summarized in Table 2 and representative cross sections of single organ innervations at various developmental stages are shown in Fig. 41. The fine structure of representative tadpole and adult innervations is shown in Fig. 42. From this study it is apparent that the

small myelinated class of fibres is first distinguishable at stage 60 and is nearly always present at stage 66. In the adult the thin myelinated fibres have been associated with efferent units (Görner, 1963; Russell, 1968). Efferent units recorded prior to stage 60 presumably represent activity in unmyelinated fibres.

Table 2

Plaque innervation at different stages of development

Stage	Number of innervations studied	Number with two afferent fibres present	Number with myelinated efferent fibre(s) present
52	4	4	0
54	6	6	0
57	15	15	0
60	9	9	2
66	11	11	10

Table 2 shows that myelinated efferent fibres are absent in larvae prior to metamorphosis, they are first seen at stage 60. Ten innervations at stage 57 were examined using the electron microscope, the rest were examined using toluidine blue-stained, thin Araldite sections and an oil immersion light microscope.

V. The development of lateral line nerves and changes in their conduction velocities

(i) Electron microscopy of early development

The development of the lateral line nerves begins rather late, the anlagen of the various lines becoming segregated from the other placodal anlagen at stage 33/34 (Nieuwkoop and Faber, 1967). By stage 45 the major features of the larval lateral line are present; nerve trunks have developed and individual organs are distinguishable. At this stage fibres begin to show signs of myelination (Figs 43A - D). Many fibres are unmyelinated, a single Schwann cell enclosing several axons. Each developing myelinated axon is encircled by one Schwann cell at any one level. In this respect the early development of the lateral line nerves resembles *Xenopus* hind limb nerve development (Peters, 1961). At the stage of limb nerve development when individual axons are first separated by glial elements coordinated limb movements first appear. It seems that the separation of axons by Schwann cells is a sign of functional maturity (Peters and Muir, 1959). Since this level of organization is present, the lateral line nerve at stage 45 is probably already functional.

(ii) Light microscopy of further development of the middle lateral line nerve trunk

By stage 49 many of the fibres in the lateral line nerve trunks have a thin myelin sheath (Fig. 44). At stage 52 the thickness of the myelin is visibly greater but small diameter

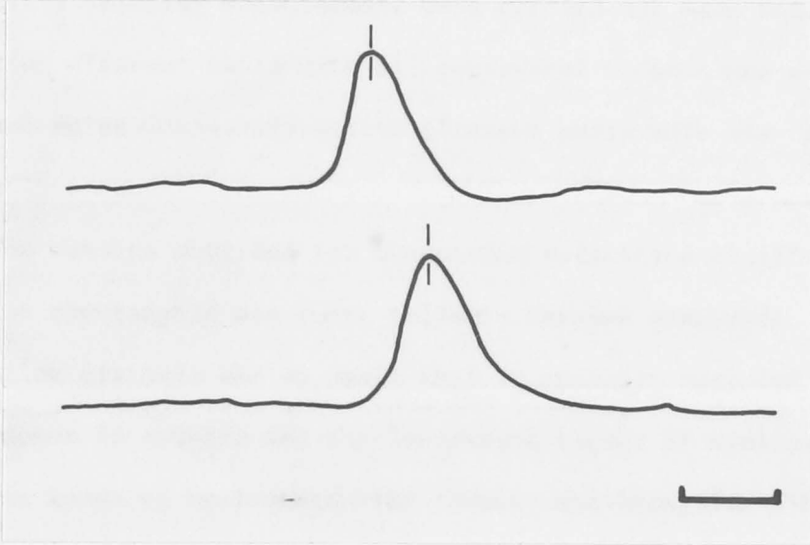
fibres with thin myelin sheaths are still present. At later stages relatively fewer immature myelinated fibres are present, the axon diameters increase and the myelin sheaths thicken (Fig. 44). The two classes of myelinated axon which can be distinguished on the basis of fibre diameters, the thick and thin fibres, only become apparent in the adult (stage 66) (Figs 44 and 45). In the larval stages, however, the presence of thin fibres could be masked by immature thick ones of small diameter. The best evidence for the absence of thin fibres comes from the examination of individual plaque innervations which shows that only two myelinated fibres innervate each larval plaque.

(iii) Conduction velocity of efferent and afferent units during development

The presence of granulated endings on the hair cell (see Chapter III) and the electrophysiological evidence of efferent activity in the tadpole (see Chapter IV) shows that efferent fibres are present before metamorphosis, but the absence of thin myelinated fibres suggests that their axons are unmyelinated. Conduction velocity measurements were made to provide supporting evidence.

All recordings were from the middle lateral line of the tadpole because it has the longest length of nerve trunk. The trunk was split and a small twig containing a few fibres was picked up. Two stiff tungsten hook electrodes mounted on the same holder were used to record activity at two places along the nerve, the operation being carried out in a humidity chamber (see Chapter IV) at 20°C. The activity from each electrode was

monitored on the upper and lower beams of the oscilloscope and recorded on moving film. The records were projected on to graph paper and the delay between spike crests measured (Text-fig. 4).



Text-figure 4

Afferent unit in a young adult recorded at two sites 1.0 cm apart. The delay indicates a conduction velocity of 15 metres per second.
Scale = 1 millisecond. (Tracing from record.)

In the tadpole the nerve is too short to record at two sites and electrically stimulate at a third. The nerve was left in place and naturally active units were recorded at the two sites. The tungsten electrodes were placed four millimetres apart for recording from the tadpole nerves and up to one centimetre apart when recording from young adults. Conduction velocity measurements were carried out upon naturally occurring efferent units with all peripheral connections of the nerve cut, and on spontaneously active afferent units with the central connections cut.

The results obtained for conduction velocities of afferent units in the tadpole are least reliable because electrode separation distance was so small that it probably included only a few nodes of Ranvier and the internodal region of myelinated axons is known to be isopotential (Huxley and Stämpfli, 1949). The same problem does not arise when recording from unmyelinated fibres but errors due to stretching the nerve apply equally to both types and are difficult to estimate. This problem is accentuated when only short lengths of nerve are available.

Afferent units have the highest conduction velocities (Fig. 46) which strongly suggests that they are the thick myelinated axons in the tadpole and adult. The increase in their average conduction velocity during development is consistent with increases in the diameters of myelinated axons seen anatomically (Fig. 46). The conduction velocities of efferent units increase considerably at metamorphosis from 1 - 4 metres per second to 4 - 12 metres per second, and correlate with the myelination of these units during metamorphosis. The evidence can only be

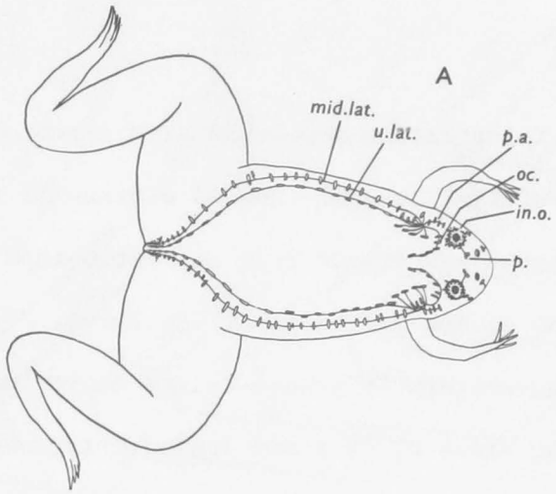
suggestive however, because of the large errors inherent in making conduction velocity measurements on such short lengths of nerve.

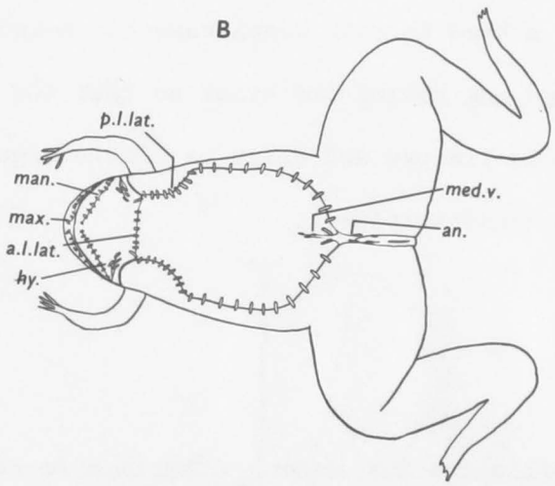
SUMMARY AND CONCLUSIONS

Peripheral reorganization of the lateral line plaques occurs at metamorphosis. This is particularly noticeable in the supra-orbital row and in this case is probably connected with the detection of surface waves by the adult. The increased angular separation of peak sensitivities of adjacent plaques in the adult supra-orbital row potentially improves its resolution. It seems likely that this sort of peripheral change would necessitate reorganization of the central nervous system. Small changes in the superficial structure of plaques occur and are related to thickening of the skin and the development of tactile receptors. They could modify the directional response. The lateral line nerves are probably functional by stage 45 and throughout subsequent development there is a gradual increase in the diameter of myelinated fibres. The thin fibres appear at metamorphosis. Efferent units can be recorded in the tadpole so probably represent activity in unmyelinated nerves. At metamorphosis there are increases in the conduction velocities of both efferent and afferent units.

Figure 35

The location of organs in adult *Xenopus* (stage 66). (A) Dorsal view, (B) ventral view, (C) lateral view, (D) arrangement of organs around the orbit. Terminology is the same as for the tadpole.


A
mid.lat.
u.lat.
p.a.
oc.
in.o.
p.


B
p.l.lat.
man.
max.
a.l.lat.
hy.
med.v.
an.

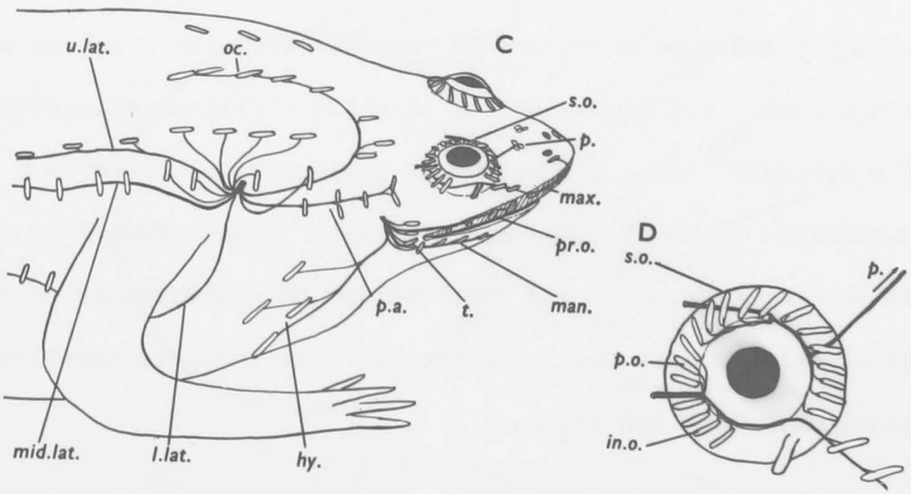

u.lat.
oc.
C
s.o.
p.
max.
pr.o.
man.
p.a.
t.
mid.lat.
l.lat.
hy.
D
s.o.
p.
p.o.
in.o.

Figure 36

The upper illustration shows the arrangement of the plaques in the supra-orbital lateral line of a stage 56 tadpole. The position of each plaque was traced from the photograph of a whole mount skin preparation of the left side of the larva. Plaque positions and their long axes are indicated by the short lines. Finer lines forming the sides of 'V's are included to show the region of maximum sensitivity of the unit in each plaque which would be excited by a head to tail displacement. Note how the plaques are arranged one behind the other so that the receptive field of the row is limited and there is little angular separation of their peak sensitivities.

The lower illustration shows a diagrammatic representation of the radially arranged supra-orbital plaques from the right side of an adult toad. All organs face a slightly different direction so that the receptive field of the row is increased and there is a separation of their peak sensitivities. This arrangement may be important for accurate localization of disturbances on the water surface (see text). Reference axes indicate anterior, posterior, left and right.

p
a
l

p
a
l

Figure 37

This photograph shows an adult *Xenopus* as it comes to the surface to breathe. Arrows indicate the positions of the orbital lateral lines and the parietal lateral line organs. They are just at the water surface while the rest of the animal with the exception of the eyes and nares is well below. The environment below the surface is outside the visual fields.

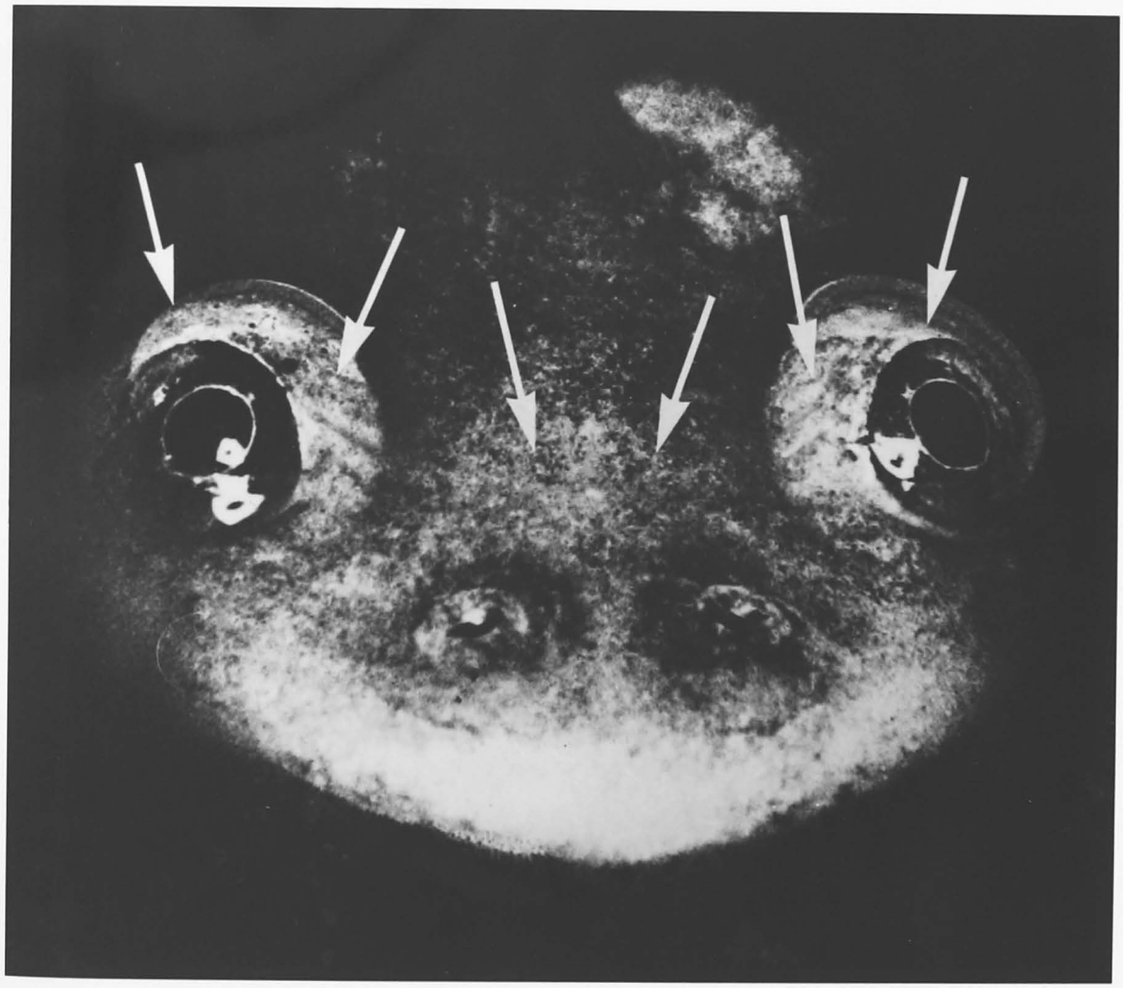


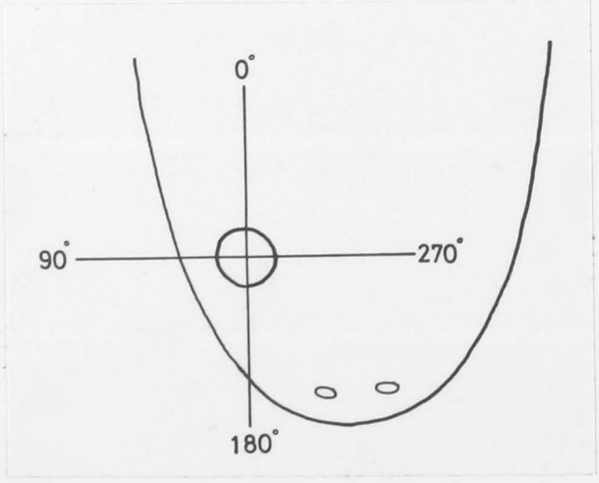
Figure 38

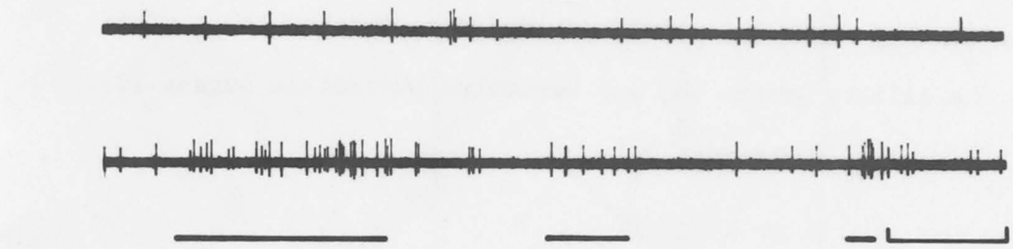
The records opposite show the spontaneous activity of afferent units in a small bundle of nerves from the supra-orbital nerve of an adult *Xenopus* at the water surface. Small movements of an insect on the surface and 10 cm from the eye (bars) cause an increase in activity.

Scale 1.0 sec.

Afferent units in the supra-orbital lateral line nerve of the adult show directional sensitivity to surface waves when the toad is at the water surface. In these records a single identifiable unit (marked with dots) fires only when the stimulating water drop hits the surface in a determined angle to the animal. The lower trace records the stimulus and the delay between the stimulus and response is due to the time taken for the wave to reach the lateral line plaques. Reference axes are shown below.

Scale 1.0 sec.





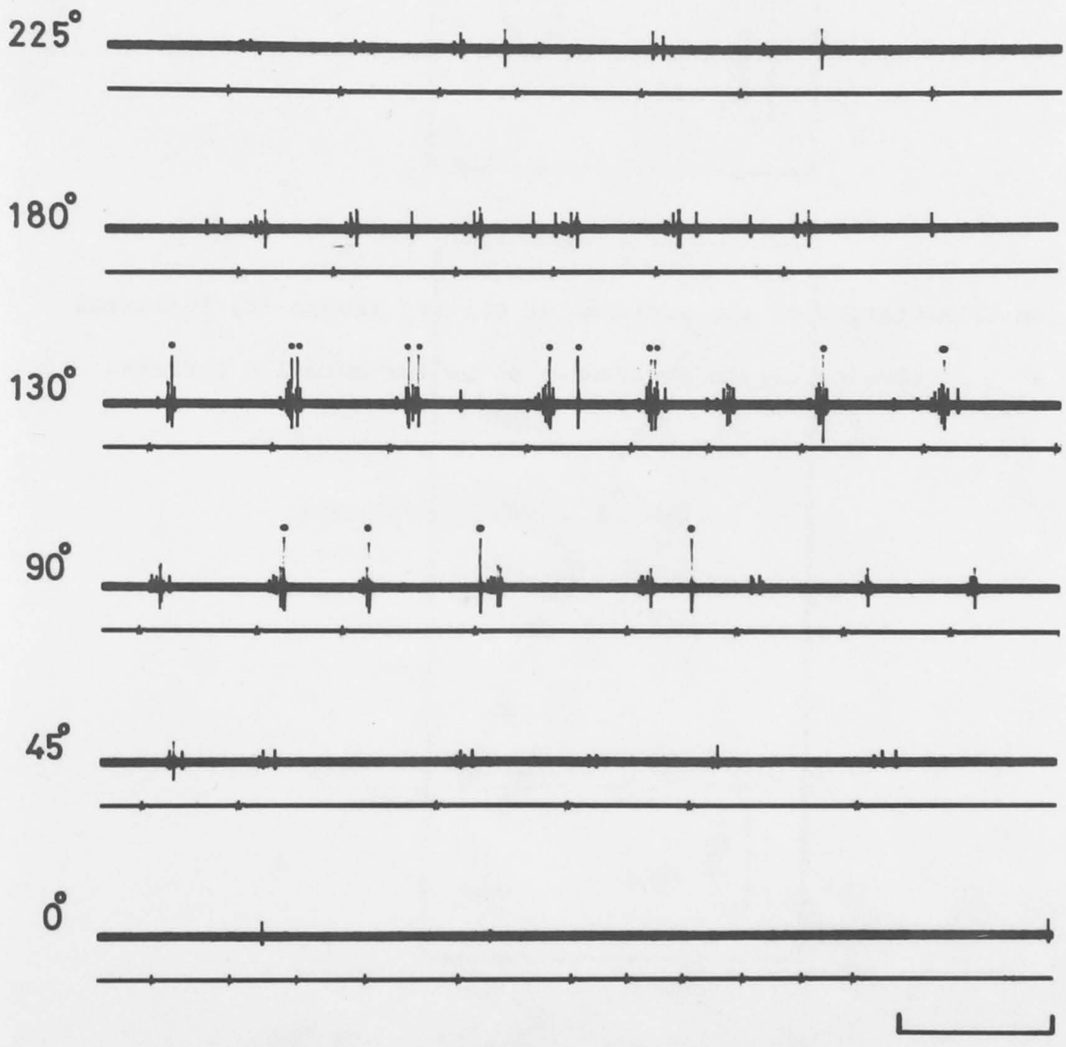
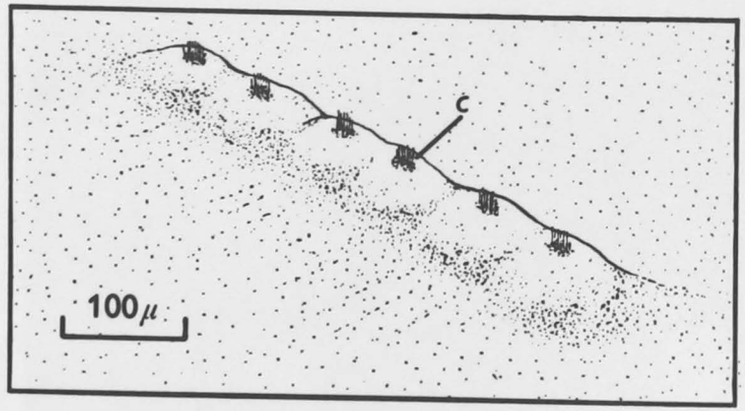

225°
180°
130°
90°
45°
0°

Figure 39

This drawing shows how the adult organs sink below the surface and the ciliary groups (c) are separated by tactile organs (t).

An illustration of the position of ciliary groups (c) in larval showing organs protruding above the animal's surface.


C
100 μ

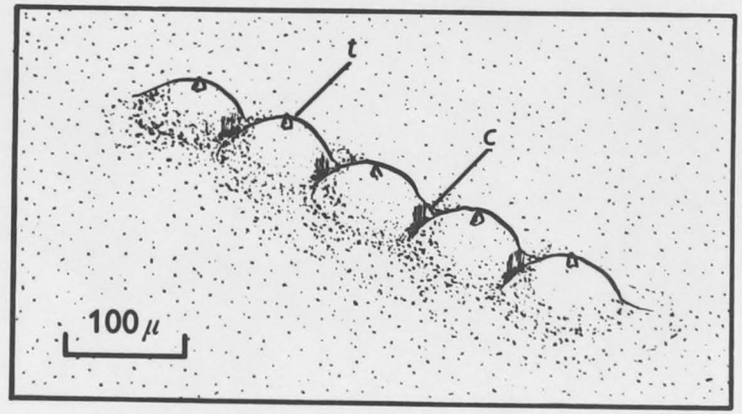

t
C
100 μ

Figure 40

Longitudinal section of an adult organ plaque showing a lateral line organ between two tactile receptors (tr). The channel formed by the two tactile organs is said to direct water currents onto the sensory hairs of the receptor cells. The densely staining cell (d) may be a degenerating cell. The material was embedded in Araldite and stained with toluidine blue. The base of the organ is richly supplied with blood vessels (bv).

Scale 15 μm.

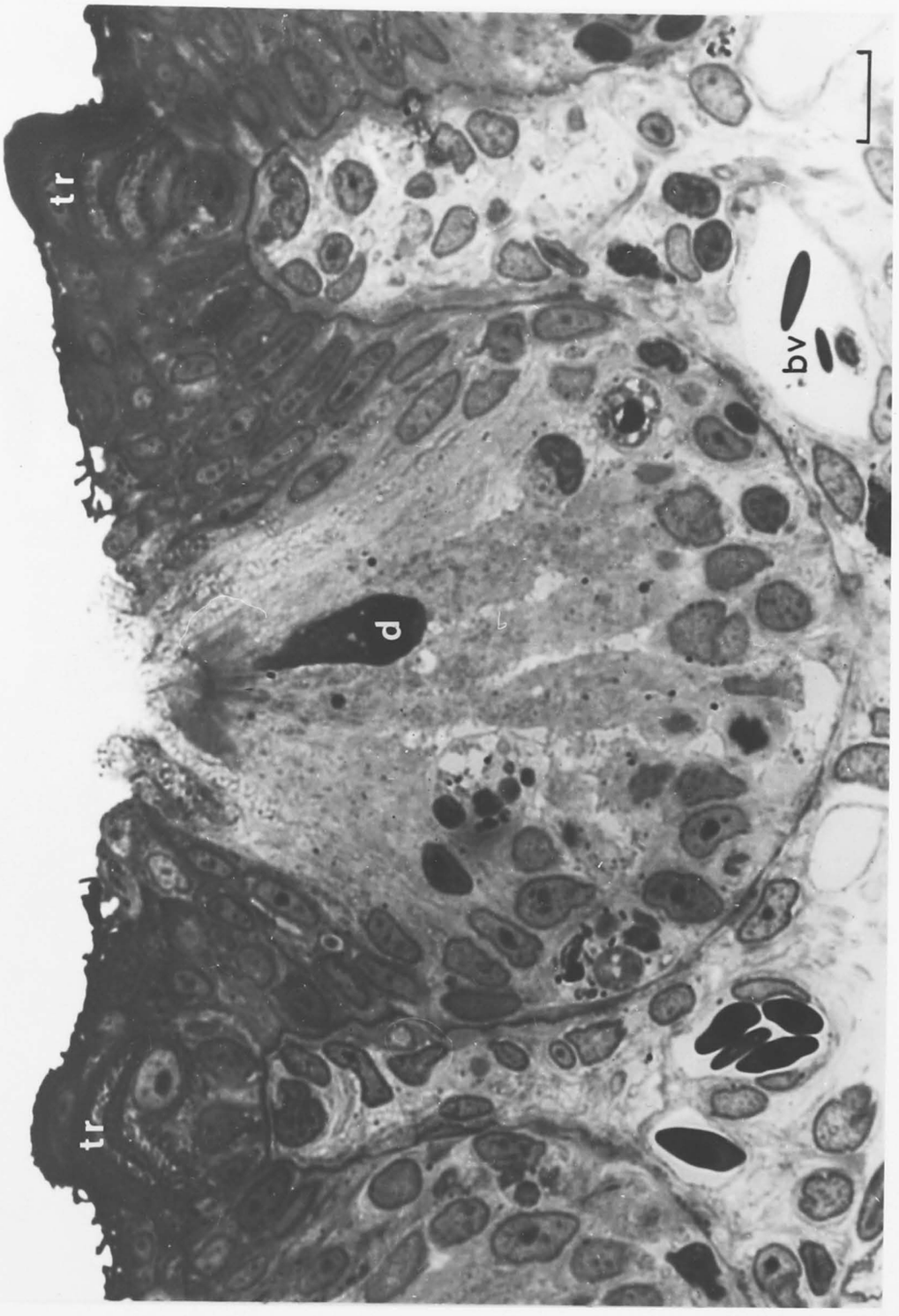

tr
tr
d
bv

Figure 41

These light micrographs show Araldite sections of single plaque innervations at various stages of development. Up to stage 60 only two myelinated fibres are present. From this stage onwards a class of smaller myelinated fibre occurs. Unmyelinated fibres are not visible in light micrographs.

The whole mount preparation on the right shows a pair myelinated axons leaving the middle lateral line nerve trunk of an adult *Xenopus*. It is interesting to note that no other nerve fibres are visible in this type of preparation. The whole mount was stained and fixed with osmium tetroxide, the sections are one micron thick and were stained with toluidine blue.

Scales: whole mount preparation 10 μm

Araldite sections 3 μm.

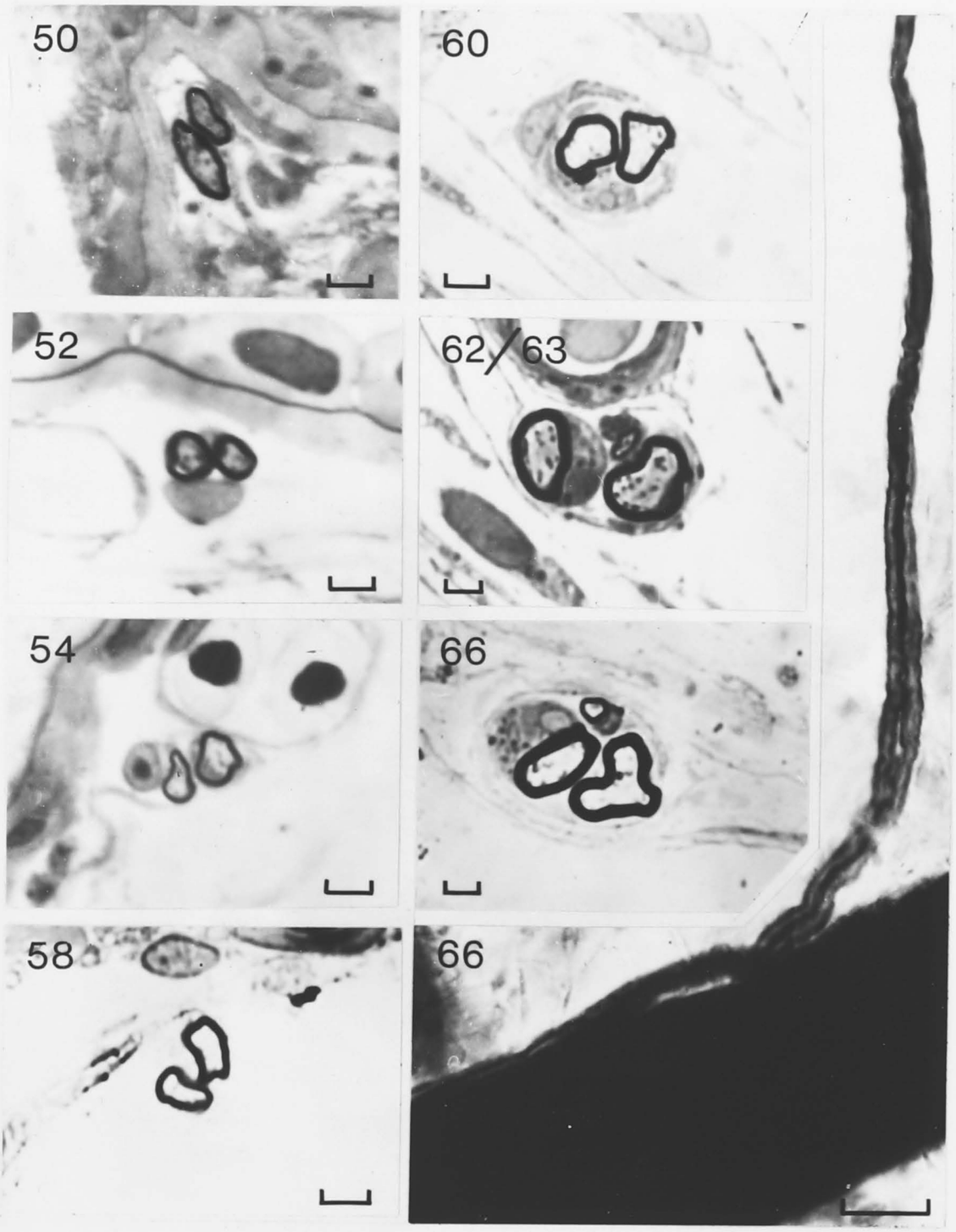

50
60
52
62/63
54
66
58
66

Figure 42

(A) An electron micrograph showing a pair of myelinated fibres (a) and a group of non-myelinated fibres (n) in the nerve bundle innervating a single tadpole plaque.

(B) An additional class of fibres is present in the adult. As well as the two myelinated afferent fibres (a), small myelinated efferent fibres (e) and often found: n = non-myelinated fibres.


A
a
n
a
2·5 μ
B
a
a
n
e
5 μ

Figure 43

These four electron micrographs of the middle lateral line nerve trunk are from a stage 45 tadpole. They show the myelination process in its early stages. Arrows show the Schwann cells (s) wrapping around the young axons (a) from stage 45 tadpoles.

Scales: A 0.5 μm

B 1.0 μm

C 0.5 μm

D 1.0 μm

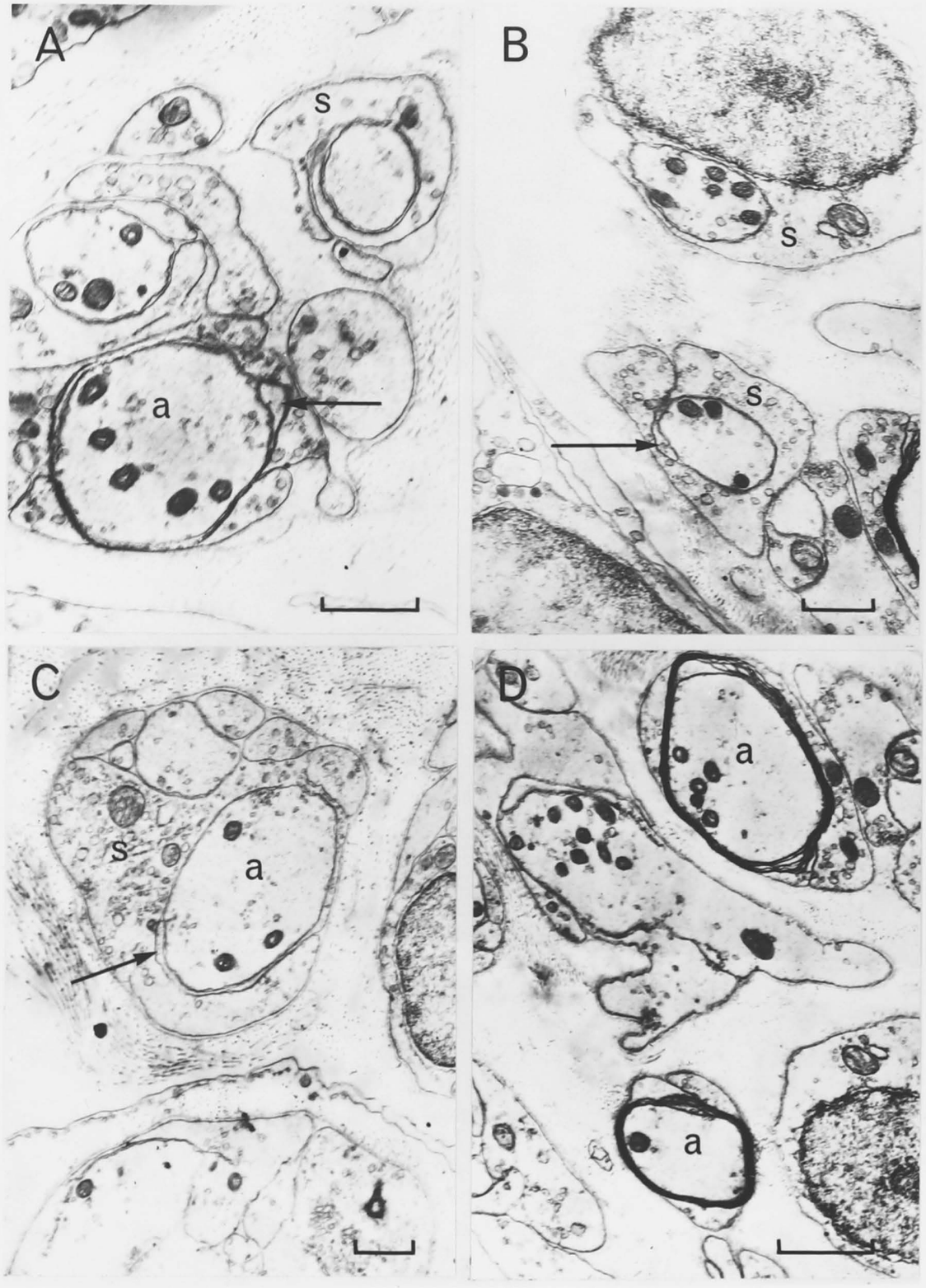

A
s
a
B
s
s
C
s
a
D
a
a

Figure 44

These six micrographs show the middle lateral line nerve trunk at various stages of development. The sections were from different animals and were taken from approximately the same region of the nerve. In younger animals there is a continuous spectrum of fibre diameters but in the adult (stage 66) two classes of fibre, large and small, are immediately distinguishable.

Scales 10 μm.

49

59

52

62/63

54

66

Figure 45

These histograms show diameters of axon profiles of the middle lateral line nerve at various stages of development. Two classes of myelinated nerve fibre (thick and thin) can be distinguished in the adult (stage 66), but not in the larval forms. This does not necessarily indicate the absence of the class of thin fibres in larvae, as their presence could be masked by developing large ones. The sections used for these counts were not from the same region of the nerve trunk, and differences in the total number of fibres in each histogram are not significant.

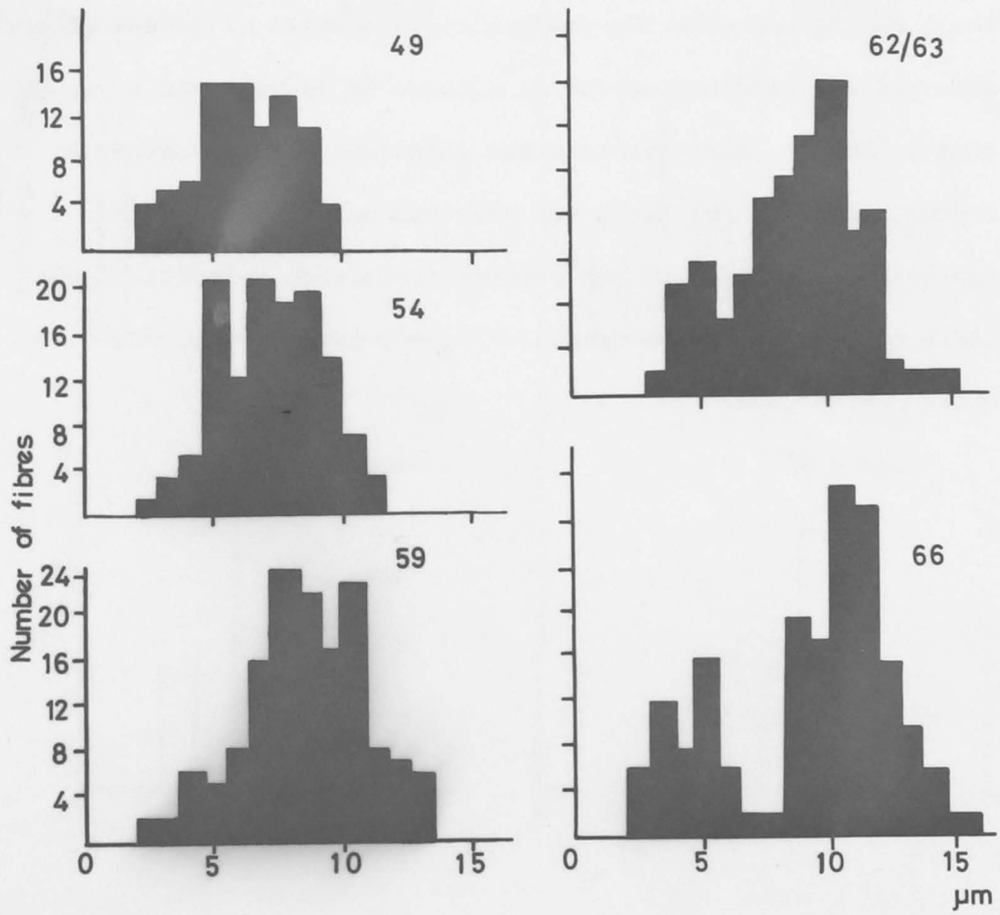
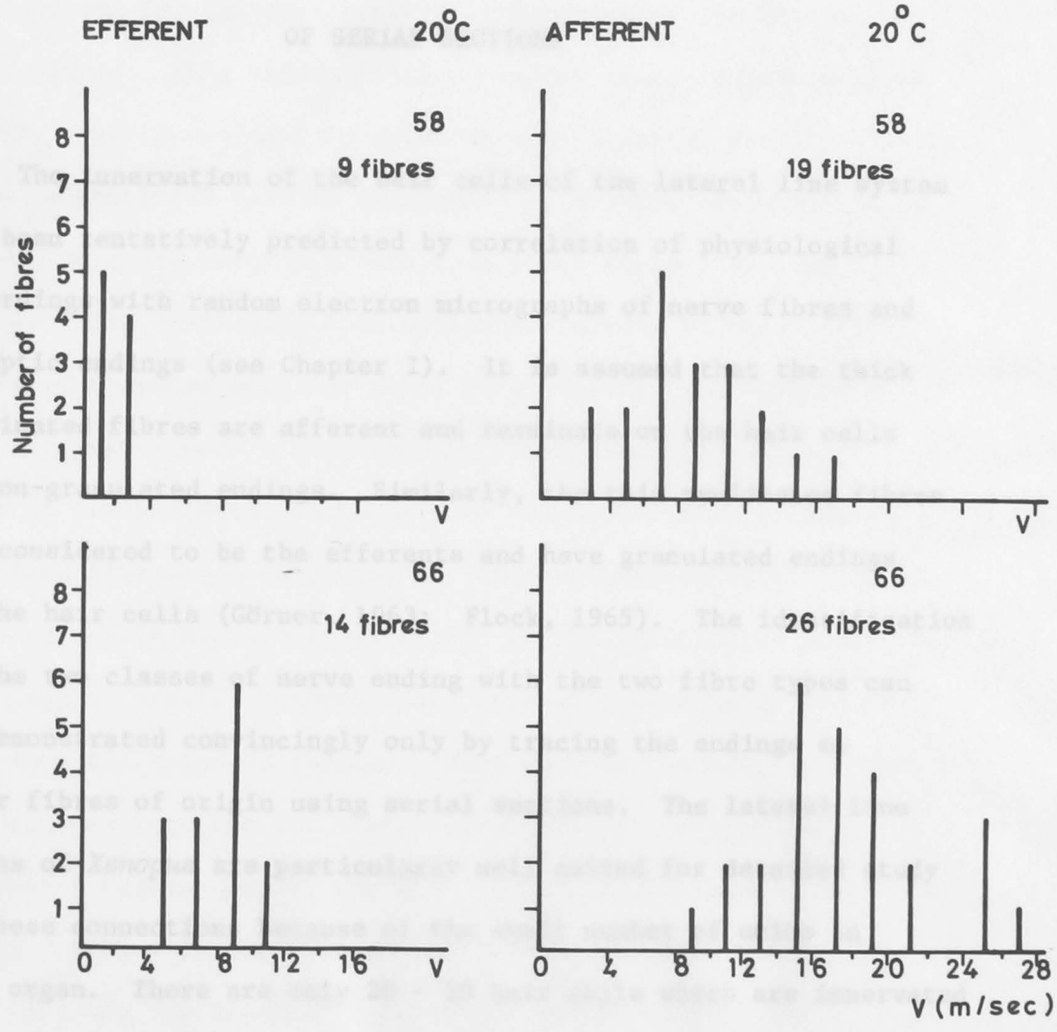

49
62/63
54
59
66
Number of fibres
16
12
8
4
20
24
0
5
10
15
µm

Figure 46

These histograms show the conduction velocities of groups of both efferent and afferent nerves in a stage 58 tadpole and a young adult. The two histograms on the left deal with the efferent fibres, those on the right the afferent ones. There is an appreciable increase in the average conduction velocity of each class of fibre at metamorphosis. V. = velocity; (m./sec.). = metres per second.


EFFERENT
20°C
AFFERENT
20°C
58
9 fibres
58
19 fibres
66
14 fibres
66
26 fibres
Number of fibres
V
V (m/sec)

CHAPTER VII

HAIR CELL INNERVATION OF THE ADULT *XENOPUS* LATERAL LINE SYSTEM STUDIED BY MEDIUM HIGH VOLTAGE ELECTRONMICROSCOPY OF SERIAL SECTIONS

The innervation of the hair cells of the lateral line system has been tentatively predicted by correlation of physiological recordings with random electron micrographs of nerve fibres and synaptic endings (see Chapter I). It is assumed that the thick myelinated fibres are afferent and terminate on the hair cells as non-granulated endings. Similarly, the thin myelinated fibres are considered to be the efferents and have granulated endings on the hair cells (Görner, 1963; Flock, 1965). The identification of the two classes of nerve ending with the two fibre types can be demonstrated convincingly only by tracing the endings to their fibres of origin using serial sections. The lateral line organs of *Xenopus* are particularly well suited for detailed study of these connections because of the small number of units in each organ. There are only 20 - 30 hair cells which are innervated by the terminal branches of a few nerve fibres only (see Chapters III and VI). In contrast, the sensory epithelium of a single lateral line canal organ of fish has many more hair cells and each organ is innervated by up to 125 myelinated fibres and an unspecified number of unmyelinated fibres (Flock, 1965). Because the thin myelinated fibres are not morphologically distinguishable

before metamorphosis (see Chapter VI) an organ from adult *Xenopus* was selected for the present study.

Görner's hypothesis that the paired thick myelinated fibres each innervate only one of the two classes of hair cell (Görner, 1963) is extremely attractive and provides a logical explanation for the dual sensory innervation of one lateral line plaque. This theory could be tested using reconstructions of the organ prepared from electron microscopy of serial sections. With conventional methods the number of sections required to trace the nerve terminals back to a point where they form anatomically distinguishable myelinated nerves is prohibitive (several thousand sections would be necessary). A technique has been developed in our laboratory for examining serial 0.25 μm thick plastic sections with the medium high voltage electron microscope (Shelton, Horridge and Meinertzhagen, 1971). With an accelerating voltage of 200 kV the penetrating power of the beam is greatly increased and chromatic aberration due to specimen thickness is reduced. This advance reduces the number of sections required for a reconstruction yet retains the necessary resolution for the identification of synaptic contacts. Using a series of 600 0.25 μm sections it was possible to identify the two classes of hair cell and to examine their connections with the thick and thin myelinated fibres.

MATERIALS AND METHODS

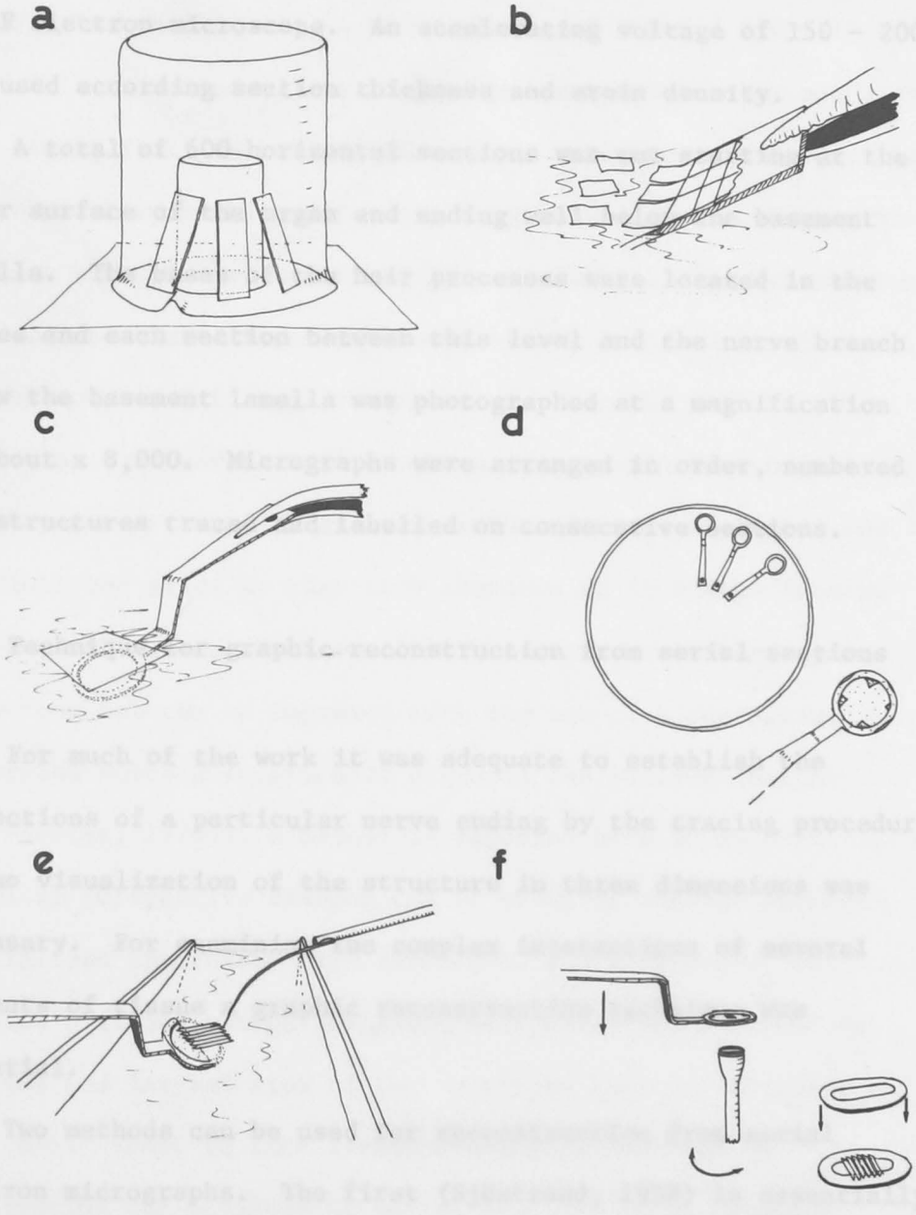
I. Preparation of the serially sectioned material

The tissue was fixed, dehydrated, block stained and embedded in plastic as for conventional electron microscopy (see Chapter III). The block was trimmed with a rectangular face approximately 1 mm long and 100 µm wide. One corner was trimmed to make subsequent orientation of the sections easy. The block was arranged with the long side parallel to the edge of the glass knife using the reflection method (Grimley, 1967). Thus the full width of the section was cut with the first sweep of the microtome arm. Several glass knives were used for cutting the series and care was taken to select good matched knives before commencing the sectioning. When the knife had to be replaced, or the block removed for trimming during cutting, realignment was carried out using the reflection method.

0.25 µm sections were cut using the manual advance of the microtome and picked up in rafts of six to eight sections on Formvar covered rings after the method of Sjöstrand (1967) (Fig. 47e). A reliable method for the preparation of Formvar covered rings is shown in Fig. 47 a - d. A 0.5 per cent solution of Formvar in dichloroethane gives a durable film which stands up to subsequent manipulation in and out of the electron microscope and gives the extra strength necessary when using large areas of unsupported film at high voltage. The sections were mounted on 2 x 1 mm slot grids (Fig. 47f) and a second grid was placed on top of the first to sandwich

Figure 47

The preparation of high quality formvar membranes for mounting serial thin sections can be difficult. The technique summarized here ensures routine production of films of uniform thickness and quality. Formvar solution is pipetted onto one side of a clean microscope slide, excess is drained off and the slide is allowed to dry for several minutes under an upturned beaker (a). Over-rapid drying results in an uneven film. To prevent this, drops of ethylene di-chloride are placed on the filter paper beneath the upturned beaker. When the microscope slides are dry the formvar surface is scored with a razor blade to produce squares of the desired size. These are floated off the slide surface onto distilled water (b), this stage is facilitated by first breathing on the formvar surface. The surface of distilled water should be illuminated with a fluorescent strip light which helps the observer to ascertain the thickness of the squares by means of its interference colour. Grey coloured films are suitable for conventional microscopy but for use in medium high voltage microscope and with thick sections pale gold films are required for added strength. Selected squares are picked up on stainless steel rings (c) and allowed to dry (d) in a petri dish placed in a warm dust-free room. The films are now ready to use and rafts of serial sections are picked up on the membrane (e). After the sections have dried onto the film they are lowered onto a two by one millimetre slot grid mounted on top of a hollow post (f). To help the film to stick, the grid can be dipped in formvar solution and allowed to dry prior to section mounting. A second grid is used to sandwich the film and prevent it sticking to the specimen holder of the microscope.


a
b
c
d
e
f

the sections in the middle. This prevents the film sticking to the specimen holder of the electron microscope while providing an uninterrupted view of the specimens. The sections were examined without surface staining and photographed in an Hitachi 200 F electron microscope. An accelerating voltage of 150 - 200 kV was used according section thickness and stain density.

A total of 600 horizontal sections was cut starting at the outer surface of the organ and ending well below the basement lamella. The bases of the hair processes were located in the series and each section between this level and the nerve branch below the basement lamella was photographed at a magnification of about x 8,000. Micrographs were arranged in order, numbered and structures traced and labelled on consecutive sections.

II. Technique for graphic reconstruction from serial sections

For much of the work it was adequate to establish the connections of a particular nerve ending by the tracing procedure and no visualization of the structure in three dimensions was necessary. For examining the complex interactions of several elements of tissue a graphic reconstruction technique was essential.

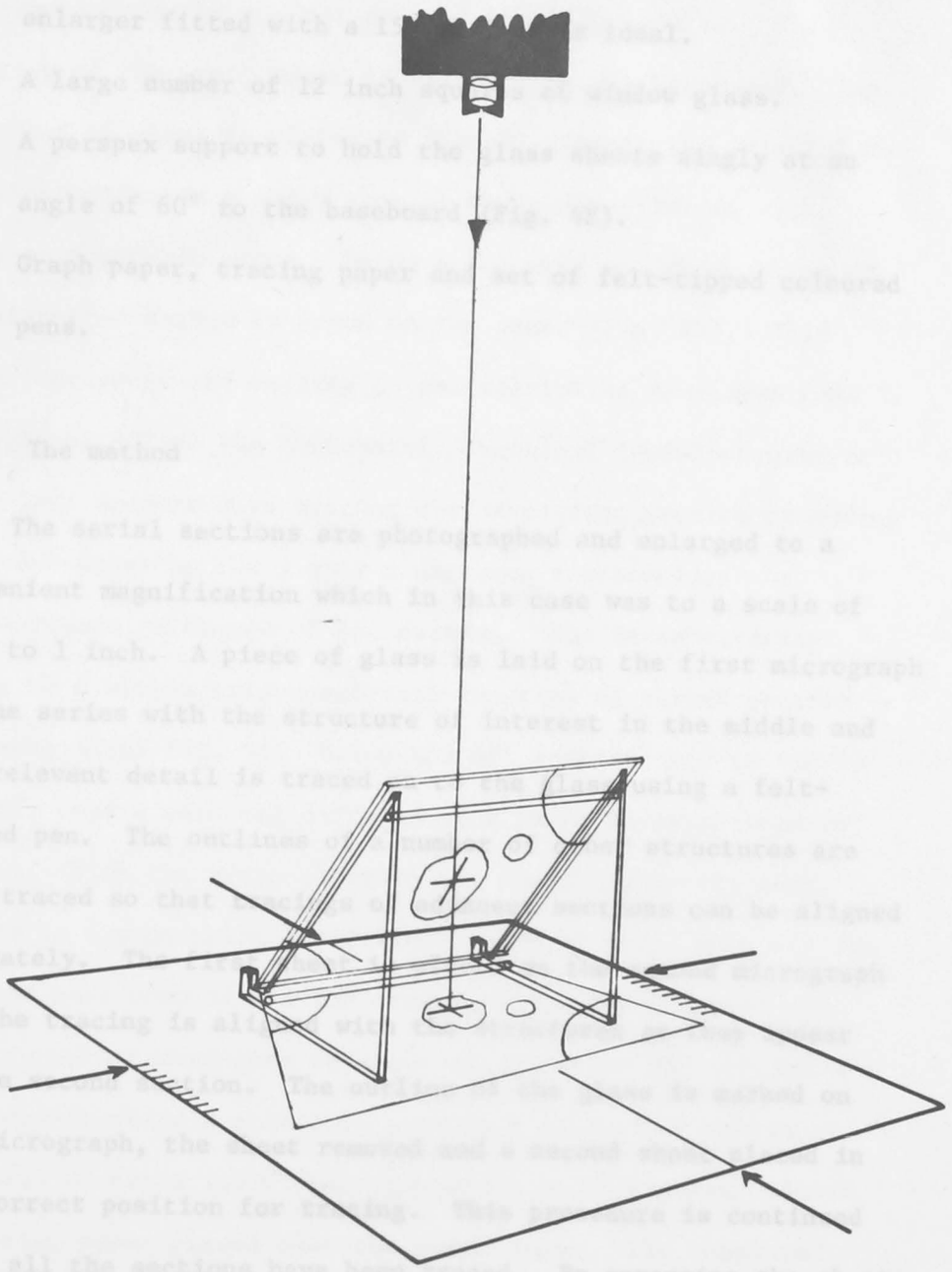
Two methods can be used for reconstruction from serial electron micrographs. The first (Sjöstrand, 1958) is essentially a modification of the solid wax model technique originally developed by light microscopists (Fell, 1936). The outlines of the structure are traced on to transparent plastic sheets which are arranged in a pile so that the structure can be seen

in three dimensions. The great disadvantage is that the model must be redrawn in 3-D for publication.

The second method of reconstruction eliminates the model building stage and produces a perspective view directly on paper. The technique is a modification of Pusey's (1939) method, but instead of superimposing contours directly one above the other, front to back distances are foreshortened and outlines of consecutive sections are displaced in one direction to give an oblique view of the structure (Barnett, 1956). The foreshortening of front to back distances is achieved by projecting sections direction onto a tilted drawing board. Various methods of achieving this effect with electron micrographs have been developed. Poritsky (1969) redrew the outlines of serial sections on distorted grids so that they appeared as if viewed from an angle of 45° to the horizontal. This method is extremely laborious but can be improved with the use of a pantograph apparatus which automatically distorts the outline (Matter and Forster, 1970). A method is reported here by which a parallel perspective drawing can be produced rapidly without specialized apparatus. The technique is a development of Barnett's (1956) method for light microscopy and produces an oblique and lateral view of the structure (see for example, Fig. 64). Front to back foreshortening is obtained by (i) tracing the outlines of serial sections on to separate sheets of glass and (ii) tilting the sheets in a beam of light and projecting the distorted images onto graph paper. The vertical displacement separating the outlines of adjacent sections is adjusted according to the desired angle of view.

Figure 48

The illustration shows details of the method of three-dimensional reconstruction developed by the author for preparing graphic models from serial electron micrographs. Structures to be reconstructed are traced from photographs onto 12 inch squares of glass. When placed in a light beam the traced structure throws a shadow on a sheet of graph paper underneath it. The glass is supported on one edge and at an angle to a light beam whose optical axis passes through the centre of the glass sheet. A distorted image of the glass is formed on underlying graph paper. Marks on the graph paper indicate x and y axes which cross at a point representing the optical axis of the light source. A reference cross also representing x and y axes is marked in the middle of the sheets of glass. The first sheet is taken and arranged so that the projected image of the cross is superimposed on the cross formed by the axes marked on the graph paper. The outline of the glass and the structure to be reconstructed is traced onto the paper. The second sheet is taken and placed on the support, the graph paper is moved up a distance representing the thickness of one section and the second image is traced. By continuing this process, a three-dimensional representation of the object is obtained.



(i) Materials

(1) A baseboard illuminated by a point source of light 24 inches above its centre - a conventional photographic enlarger fitted with a 150 mm lens is ideal.

(2) A large number of 12 inch squares of window glass.

(3) A perspex support to hold the glass sheets singly at an angle of 60° to the baseboard (Fig. 48).

(4) Graph paper, tracing paper and set of felt-tipped coloured pens.

(ii) The method

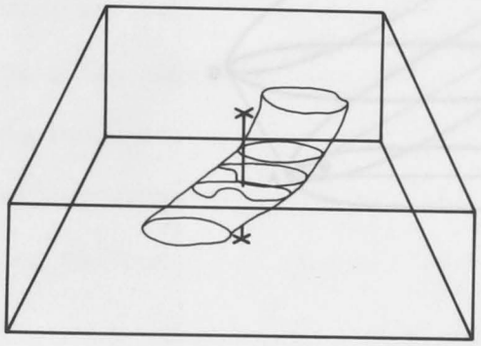
The serial sections are photographed and enlarged to a convenient magnification which in this case was to a scale of 1 μm to 1 inch. A piece of glass is laid on the first micrograph of the series with the structure of interest in the middle and the relevant detail is traced on to the glass using a felt-tipped pen. The outlines of a number of other structures are also traced so that tracings of adjacent sections can be aligned accurately. The first sheet is placed on the second micrograph and the tracing is aligned with the structures as they appear in the second section. The outline of the glass is marked on the micrograph, the sheet removed and a second sheet placed in the correct position for tracing. This procedure is continued until all the sections have been traced. By arranging the sheets in a pile the three dimensional structure can be visualized.

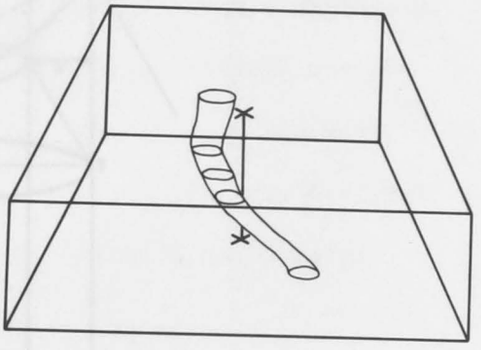
The next stage of the operation is concerned with foreshortening front to back distances and transferring the outlines on to paper.

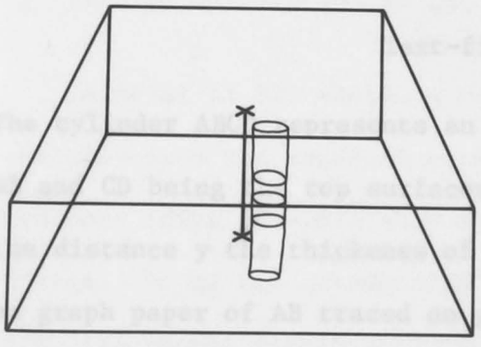
The 12" x 12" glass sheets are marked with x and y axes parallel to the edges and bisecting each other in the centre of the sheet. In the same way, x and y axes are marked on a sheet of graph paper which is placed on the baseboard with the point of axis intersection on the optical axis of the light source. The location of the graph paper is recorded on the baseboard. The top sheet of glass is placed on the 60° support and adjusted so that the projected x and y axes on the glass coincide with those on the graph paper. Using a coloured pen the outline of the distorted shadow is drawn on the paper (Fig. 48). This image represents the outline of the section as if viewed from an angle of 30° to the horizontal. Vertical foreshortening is taken into account when tracing the subsequent section by moving the graph paper up the y axis a distance representing the foreshortened thickness of one section. The foreshortening factor is a simple trigonometrical function of actual section thickness (Text-fig. 5). Using the 60° support this is Sin 60° or 0.86, thus a vertical distance of 1 μm is foreshortened to 0.86 μm. With the graph paper in the new position the next outline is traced using a different coloured pen. When all the sections have been traced the diagram shows the contours of the structure viewed from an angle of 30° below the horizontal. For reconstructing a structure such as a network of nerves it is useful to trace individual components on to separate sheets of tracing paper placed over the graph paper. The completed tracings are arranged in a pile and the whole structure can be visualized (Fig. 49). This method is particularly useful where one structure lies behind another because it eliminates two superimposed sets of contours on the same diagram.

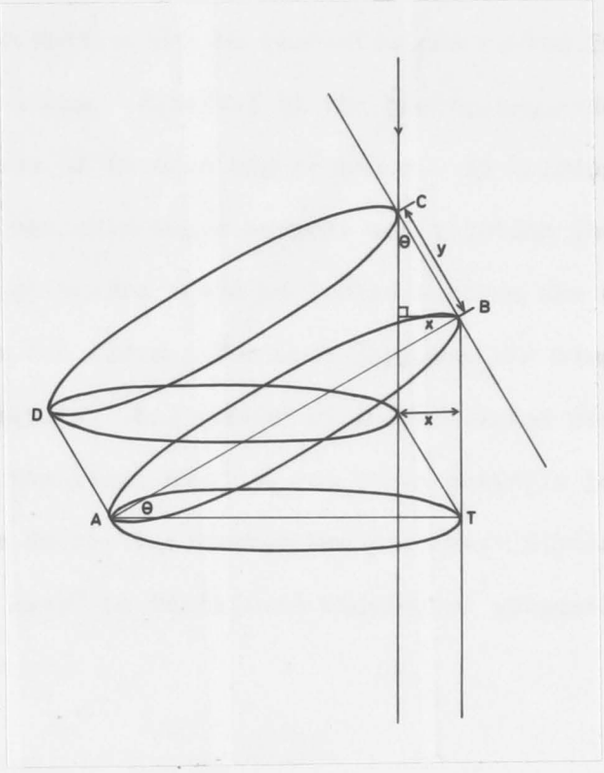
Figure 49

This composite figure shows how a complicated structure can be reconstructed. The three different structures are traced onto separate sheets of tracing graph paper. Then the separate sheets of tracing paper are superimposed and the final diagram is traced onto ordinary tracing paper.









Text-figure 5

The cylinder ABCD represents an element of the tissue investigated, AB and CD being the top surfaces of adjacent serial sections, the distance y the thickenss of one section. AT is the projection on graph paper of AB traced on glass. The angle $B\hat{A}T$ is the angle of inclination θ of the glass to the graph paper. The graph paper is moved a distance x for successive serial sections, such that $x = y \sin \theta$. The reconstruction appears as if viewed from an angle = $(90 - \theta)^\circ$ from the horizontal.

Using the apparatus described many views of an object can be obtained. By resting the glass sheets on different edges, the structure can be reconstructed viewed from any of the four sides. Reversal of the projection order of the sections shows it from on top or below. By holding the glass sheets in a variable angle support and rotating them in the holder, the structure could be looked at from the corners as well as from the sides. For most purposes the simple apparatus is quite adequate. Any amount of intracellular detail can be included in the final diagram and it is possible to add more at any stage in the reconstruction process. Similarly features can be "cut away" to facilitate viewing of internal detail.

RESULTS

I. General description of the organ and section series

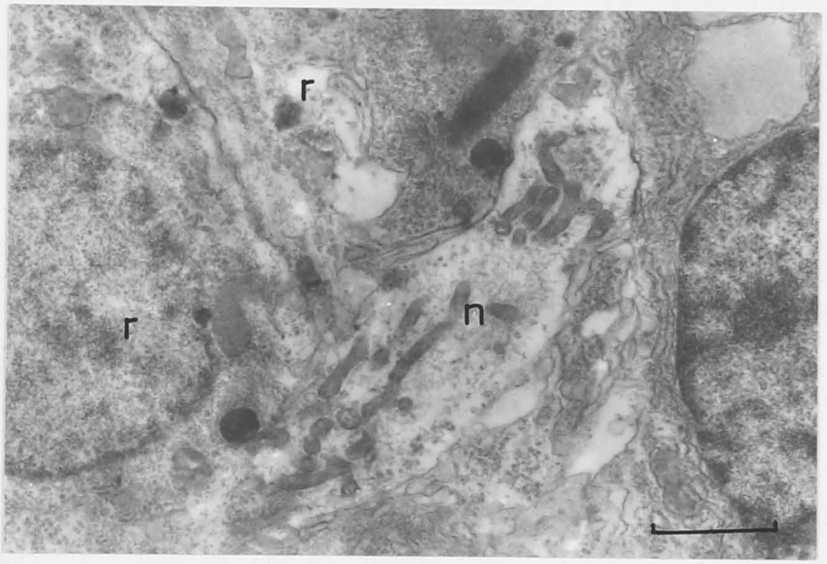
A total of 525 sections was photographed and numbered serially from the external surface of the organ. Representative sections (Figs 50 - 62) show the morphology of the organ at different levels. Using the asymmetry of the hair process as the criterion for directional sensitivity (see Chapter III), two types of hair cell could be distinguished (Fig. 51). Of 23 identified hair cells, 11 were of one class and 12 of the other. Cells were given arbitrary identification numbers and their directional sensitivities were indicated on photographs using arrows to show the direction of hair process deflection which is assumed to result in cell depolarization (Lowenstein and Wersäll, 1959).

Four large (thick) and two small (thin) myelinated nerve fibres were identified in the innervation of the plaque to which the organ belonged (Fig. 62). These fibres were followed from section to section but unmyelinated fibres could not be distinguished clearly in all sections and were therefore untraceable. Two of the six myelinated fibres (designed α and β) and both of the thin fibres (designated γ and δ) sent branches to the organ examined. The other two thick fibres were almost certainly branches of fibres α and β and innervated another organ in the plaque. Terminals of fibres α, β and γ were identified on hair cells but fibre δ could not be traced within the organ.

II. The innervation

(i) Non-granulated endings

The base of each receptor cell is contacted by a single non-granulated ending which contacts the base like a hand holding an orange (Fig. 63). There is never more than one non-granulated ending on each receptor cell but one ending may enclose the bases of two adjacent cells (Text-fig. 6). Each non-granulated ending has many points of synaptic contact with its hair cell. These are identifiable by the presence of synaptic bars lying close to the receptor cell membrane. The total number of synapses is different from one receptor cell to another. Counts for cells 11, 13, 18 and 22 revealed 51, 46, 37 and 29 synapses respectively. It was possible to follow the nerve endings associated with nine receptor cells to their fibres of origin. Of these, five cells polarized in



Text-figure 6

Electron micrograph showing one non-granulated ending (n) contacting two adjacent receptor cells (r).
Scale = 0.5 μm.

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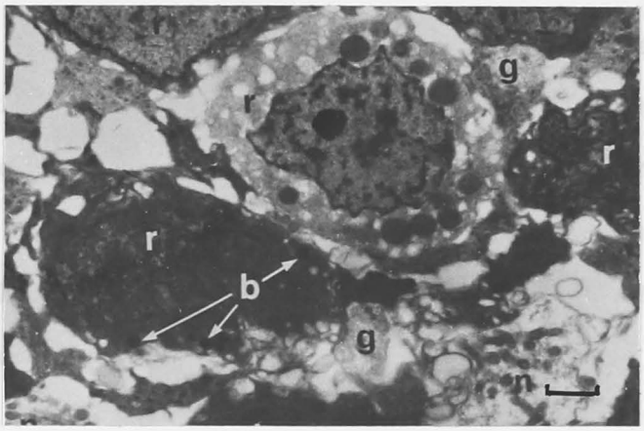
one direction were innervated by fibre α and four with opposite polarity by fibre β.

(ii) Granulated endings

There appears to be one granulated ending associated with the base of each hair cell (Text-fig. 7). These endings are much smaller than the non-granulated terminals and each has only one point of synaptic contact with its receptor cell. In several



Text-figure 7

Two receptor cells (r) in the centre of the electron micrograph each associated with a single granulated ending (g).
Scale = 1.0 μm.

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cases synaptic contacts between granulated and non-granulated terminals were observed. These are characterized by the presence of a cistern within the non-granulated terminal and they resemble the synaptic contacts between granulated endings and hair cells.

The granulated ending associated with cell 13 was traced to the thin fibre γ below the basement lamella (Fig. 62). Attempts to trace other granulated terminals to their fibre of origin were unsuccessful because of their tortuous pathways and small diameters.

Cell 13 and its nerves were examined in detail and reconstructed graphically (Fig. 64). The non-granulated ending which contacts cell 13 is a terminal of the thick fibre β. The granulated ending consists of a branching tree-like arrangement of processes. This ending has two morphologically identifiable synaptic contacts (Fig. 65), one with the non-granulated ending (Fig. 66) and one directly with the hair cell (Fig. 67). Neither of these synapses is of the terminal bouton variety; both are *en passant* contacts. The destinations of the branches 1 - 5 (Fig. 64) of the granulated ending were not traced.

III. Observations on the different cell types in the organ

(i) Receptor cells

The receptor cells are of different shapes and sizes (compare cross sections of cells 1 and 2 in Fig. 54) and they vary in the density of their cytoplasmic contents. Some of the cells, e.g., 2, 8, 12 and 13, have densely staining cytoplasm and some, e.g., 1, 8 and 16 have large cysternae of the endoplasmic reticulum and large mitochondria (Figs 53 and 54).

All of the receptor cells examined contained dense inclusion bodies (Fig. 55) not previously described in conventional micrographs. They resemble bodies found in the receptor cells of ampullary electric receptors (Mullinger, 1964). Part of cell 20 was reconstructed to examine the distribution of these bodies (Figs 68 and 69). Each cell has one or two inclusions which are much larger than the rest. These are often square or triangular in cross section and are 1 - 2 μm wide at the broadest point. A

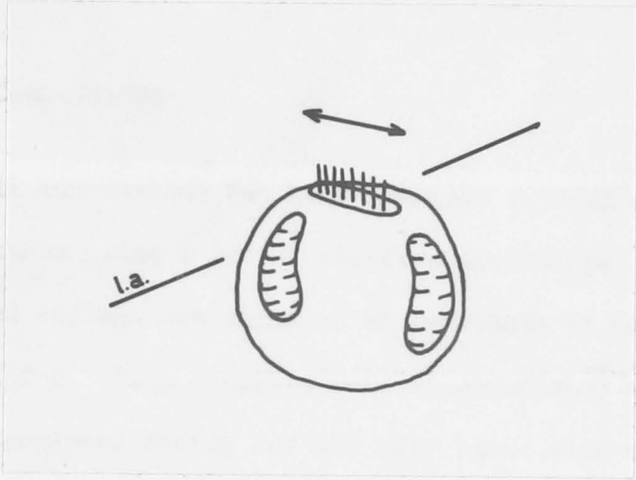
number of smaller dense bodies (0.1 - 1.0 μm in diameter) are closely associated with the larger inclusions and many more are scattered in a layer around the top of the nucleus. These smaller inclusions could be stages in the formation or disintegration of the larger ones. There is an obvious superficial resemblance between these dense inclusions and synaptic bars but without more substantial evidence a functional correlation would be unwarranted at this stage.

(ii) Undifferentiated cells

Two tightly packed groups of cells are situated on either side of each organ (Text-fig. 8 and Figs 55 - 58). Each of these cells has the same shape as a receptor cell, tapering towards the top of the organ, but none reaches the outer surface of the animal. Their function is unknown but they could be immature receptor cells. Turnover of receptor cells is well known in taste buds (Beidler and Smallman, 1965; Conger and Wells, 1969) and it would not be surprising to find a similar replacement of receptor cells in lateral line organs. Both taste buds and lateral line organs are superficial receptors and they would be subject to considerable wear and erosion.

(iii) Supporting cells

The observations on the supporting cells confirm earlier reports (see Chapter III). Features of interest are mentioned in the Figure subscripts (Figs 50 - 62).



Text-figure 8

Groups of undifferentiated receptor cells occur on each side of the organ. The plaque's long axis is marked (l.a.) and the directional sensitivity of the organ is indicated.

IV. Tactile receptor innervation

At metamorphosis, tactile receptors develop between the individual organs in each plaque (see Chapter VI). Murray (1955) showed that these receptors are innervated by separate cutaneous nerves. Electron micrographs show that a large number of naked nerve fibres terminate at the base of each tactile receptor (Fig. 70A). Examination of serial sections confirmed

that these fibres join small bundles of cutaneous nerves (Figs 70b - c) and not the lateral line nerve. It is therefore certain that unmyelinated fibres in the lateral line nerve do not contribute to the innervation of the tactile receptors.

SUMMARY AND CONCLUSIONS

Hair cell innervation has been examined in 0.25 μm serial Araldite sections using a 200 kV electron microscope. The non-granulated endings are shown to be terminals of thick myelinated fibres. Each receptor cell is associated with a single non-granulated ending and all hair cells with the same morphological polarization of the hair process are innervated by branches of the same thick fibre. This explains the dual sensory innervation of a single plaque and confirms Görner's (1963) theory of hair cell innervation.

Using a new graphic reconstruction method it has been established that thin myelinated fibres end in granulated terminals. Granulated endings make synaptic contact both with the hair cells and with the non-granulated terminals.

The unmyelinated fibres of the lateral line nerve could not be traced into the organ. Their final destination is unknown but they cannot be associated with the tactile receptors as these are separately innervated by the cutaneous nerves.

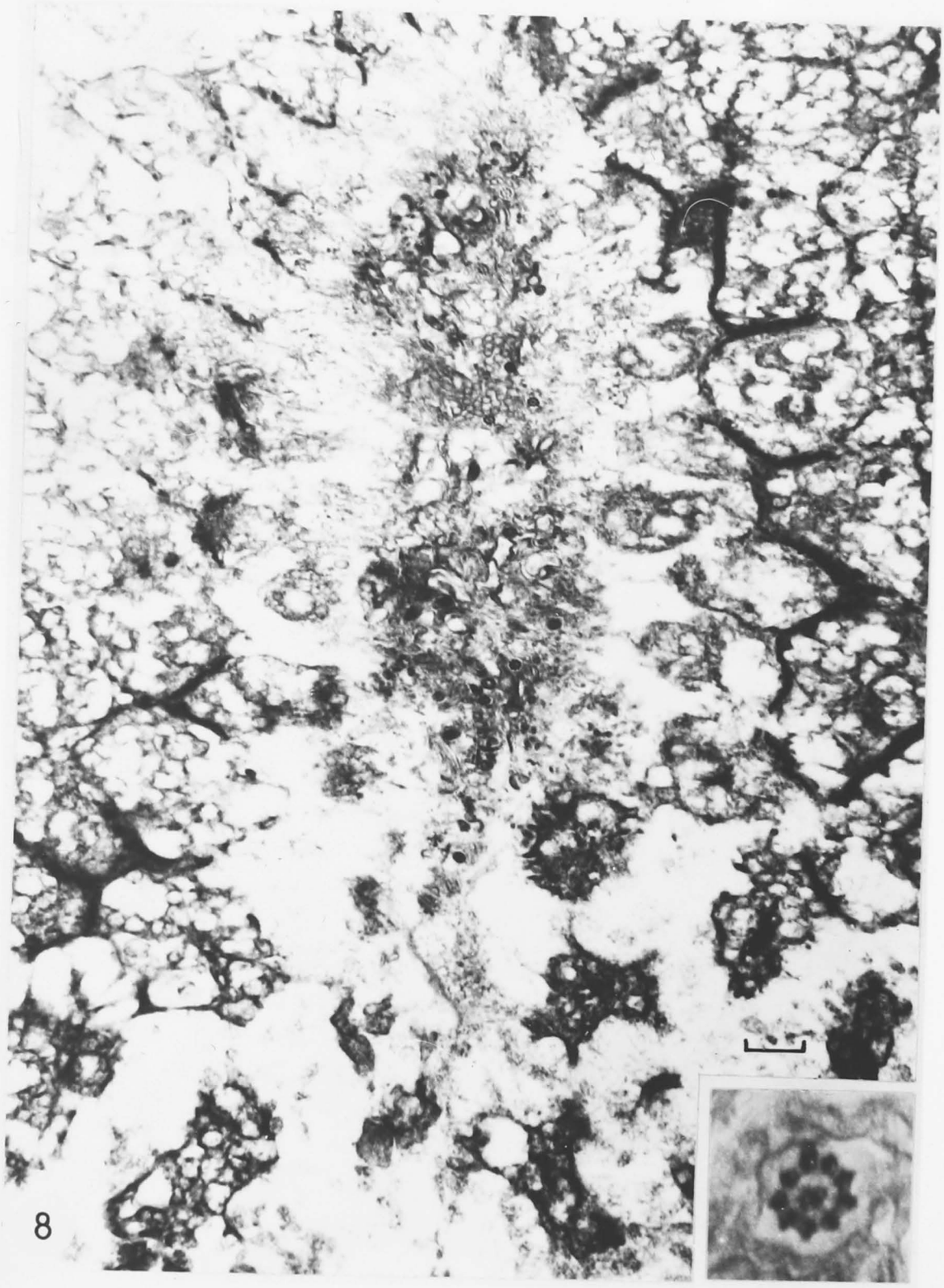
The hair cells show great variation in size and in density of cytoplasm. Such differences are consistent with the theory that there is a cyclic renewal of receptor cells, which is further supported by the presence of undifferentiated cells at the sides of the organ.

Dense inclusion bodies of unknown function are described in lateral line hair cells; similar bodies are already known in the receptor cells of ampullary electric receptors (Mullinger, 1964).

Figure 50

The following fourteen figures are representative sections from a series of 600 horizontal sections through an adult lateral line organ and its innervation. They show detail resolvable at various levels of the organ. A receptor cell is thought to be depolarised when the hair-cell bundle is bent towards the side marked by the kinocilium. On this basis there are two classes of cell in each organ which are depolarised by opposite deflections of the hair-cell bundles. The 23 receptor cells considered in this study are marked with an arrow to indicate the deflection direction resulting in depolarisation. Of these 23 cells the non-granulated endings of 10 were traced to nerve fibres below the basement lamella. In the same way the granulated endings associated with cell 13 were traced to a nerve fibre in the innervating bundle. The sections were approximately $^{1}/_{5}$th to $^{1}/_{4}$ μm thick and were examined with an Hitachi Hu 200F electron microscope. Serial sections were numbered in order, those with the lowest numbers being nearer the top of the organ. In the following illustrations the section number is shown in the bottom left-hand corner of each micrograph.

This section shows kinocilia and stereocilia just above the cuticular plate region of the receptor cells. The receptor cells are surrounded by highly vacuolated supporting cells.
Scale 1.0 μm.
The inset shows resolvable detail of a kinocilium at higher magnification.

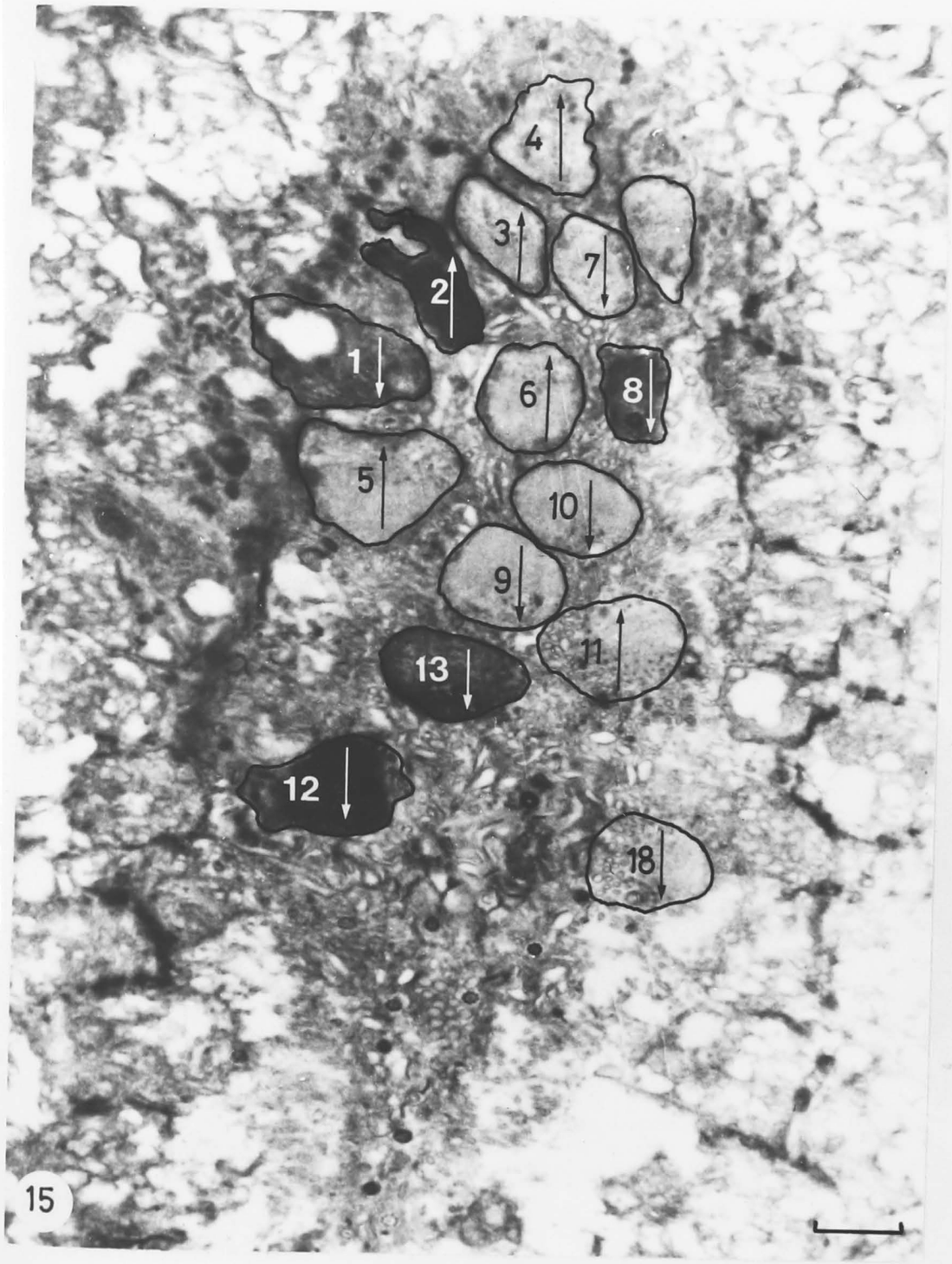


8

Figure 51

This micrograph of the top of the organ is just less than two microns below the previous one. Arrows on the numbered receptor cells indicate directions of cupular displacement which would result in depolarisation. Criteria used for identifying this directionality can be seen for cells eight and 18; the kinocilium is to one side of the cuticular plate and stereocilia. Displacement of the hair-cell bundle towards the kinocilium is said to cause depolarisation of the receptor cell.

Scale 1.0 μm.



15

Figure 52

More sense cells can be identified at this level. Supporting cells are packed between the receptor cells and isolate each one from its neighbours.

Scale 1.0 μm.

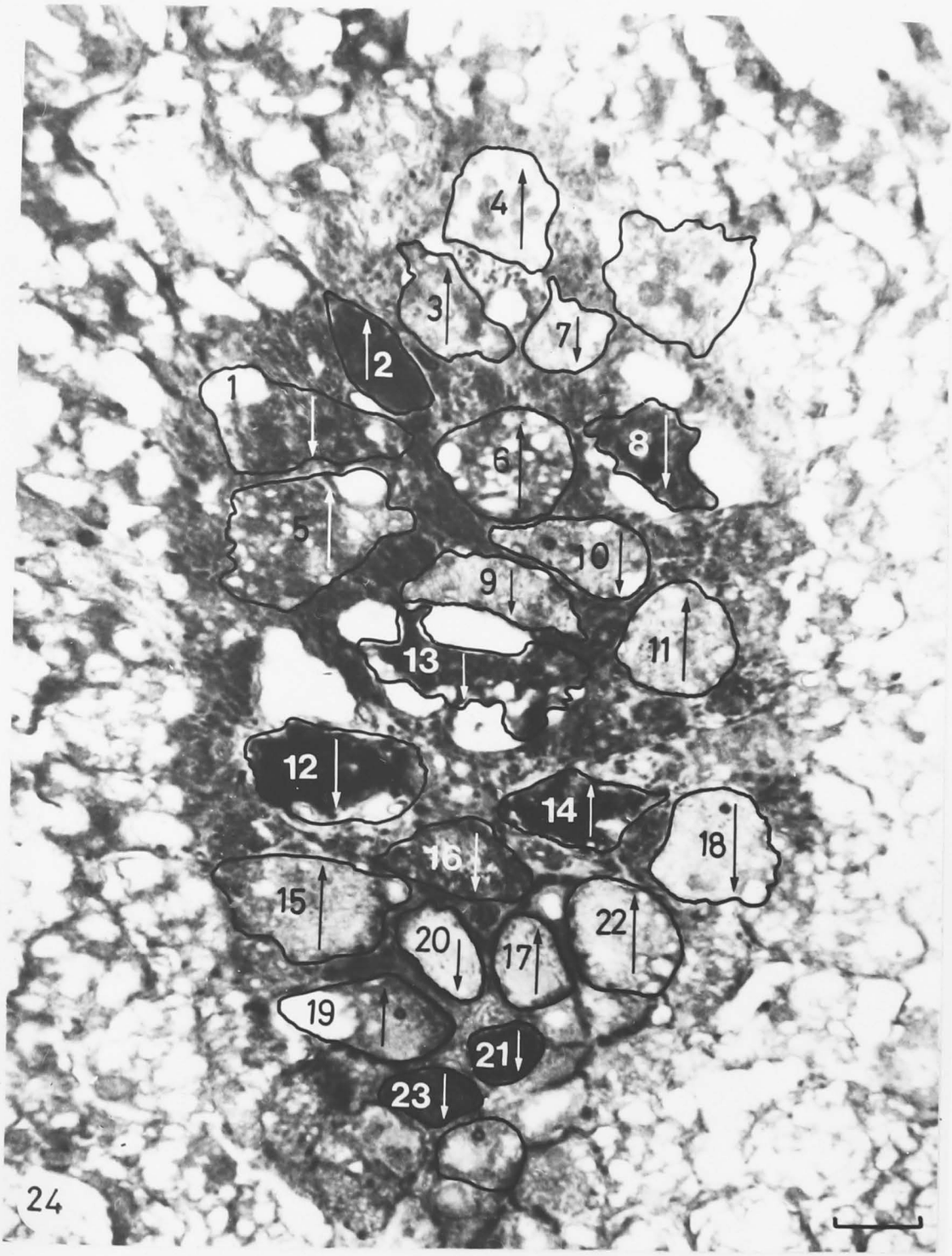

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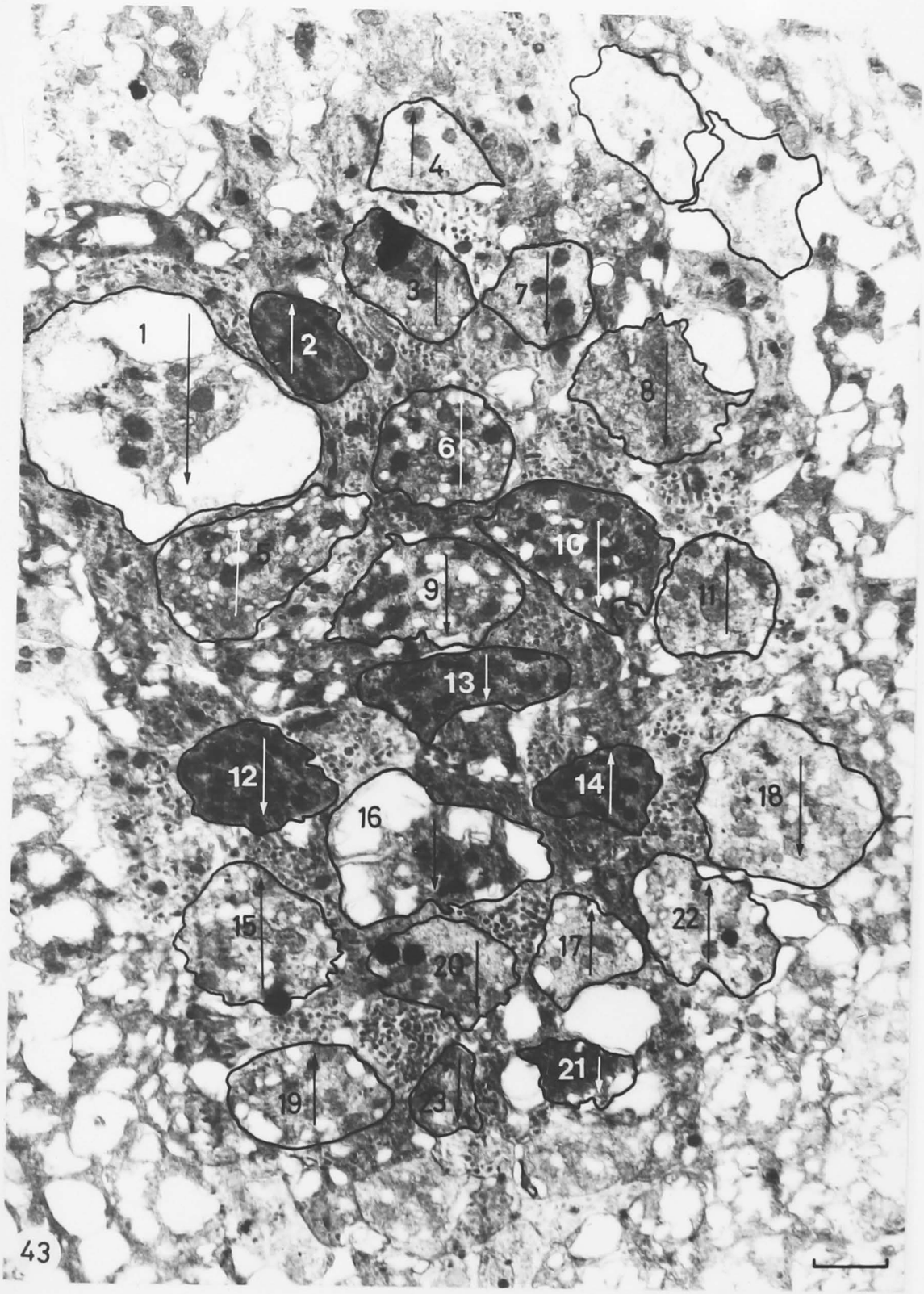
Figure 53

This section is from a level just below the cuticular plate regions of the receptor cells. Supporting cells have granular inclusions and still isolate the sense cells. Cells 1 and 16 have large cysternae of the endoplasmic reticulum and the receptor cells are of varying electron-densities.
Scale 1.0 μm.

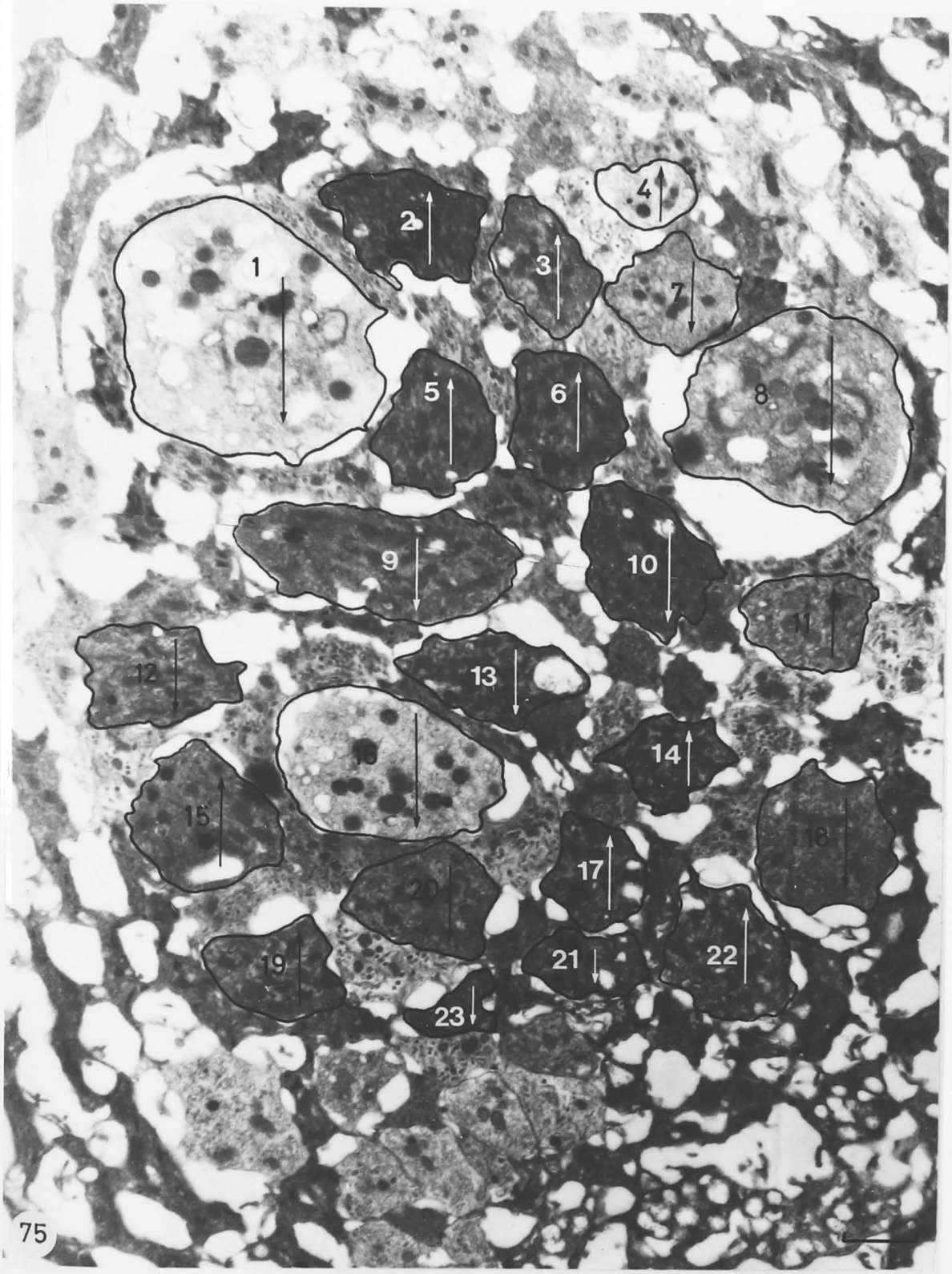


43

Figure 54

This section is from a level seven to nine microns below the cuticular plate. There is considerable variation in receptor cell size. Cells one and 16 contain large mitochondria. The two groups of small cells at the upper and lower poles of the micrograph may be developing receptor cells. Note the occurrence of intercellular space at this level.

Scale 1.0 μm.

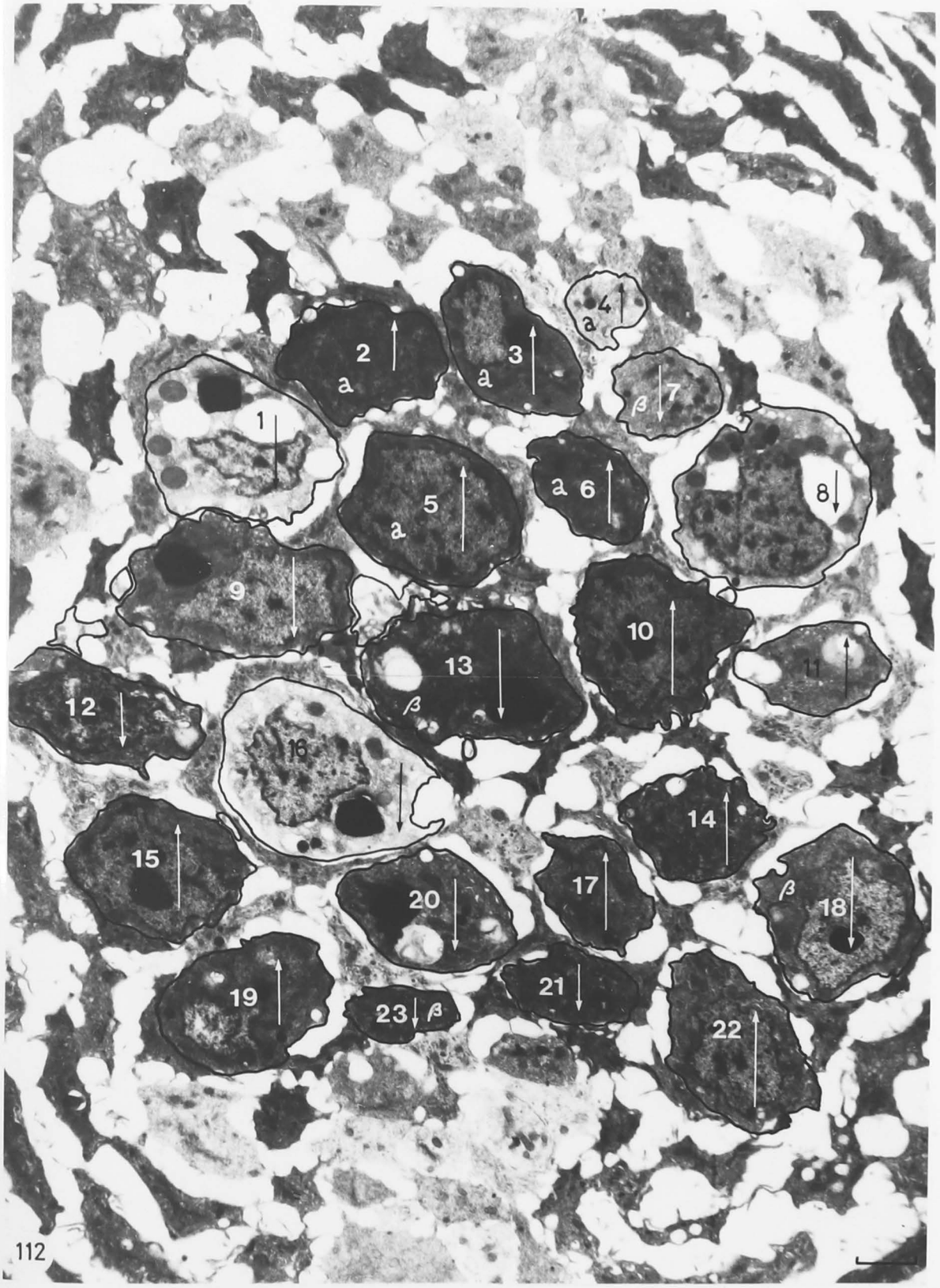


75

Figure 55

This micrograph is at the level of the tops of the receptor cell nuclei. Dense inclusions are visible in cells 1, 3, 9, 13, 16, 18 and 20. All receptor cells have at least one of these bodies, they never occur in supporting cells. A few nerve processes are visible in this section.

Scale 1.0 μm.



112

Figure 56

This micrograph from a region approximately 25 to 30 μm below the cuticular plate shows the terminals of nerve fibres. Those which could be traced to identified nerve fibres below the basement lamella are marked with Greek letters. The terminals marked α and β are branches of two large myelinated fibres and those marked γ and δ the terminals of two small myelinated fibres. Scale 1.0 μm.

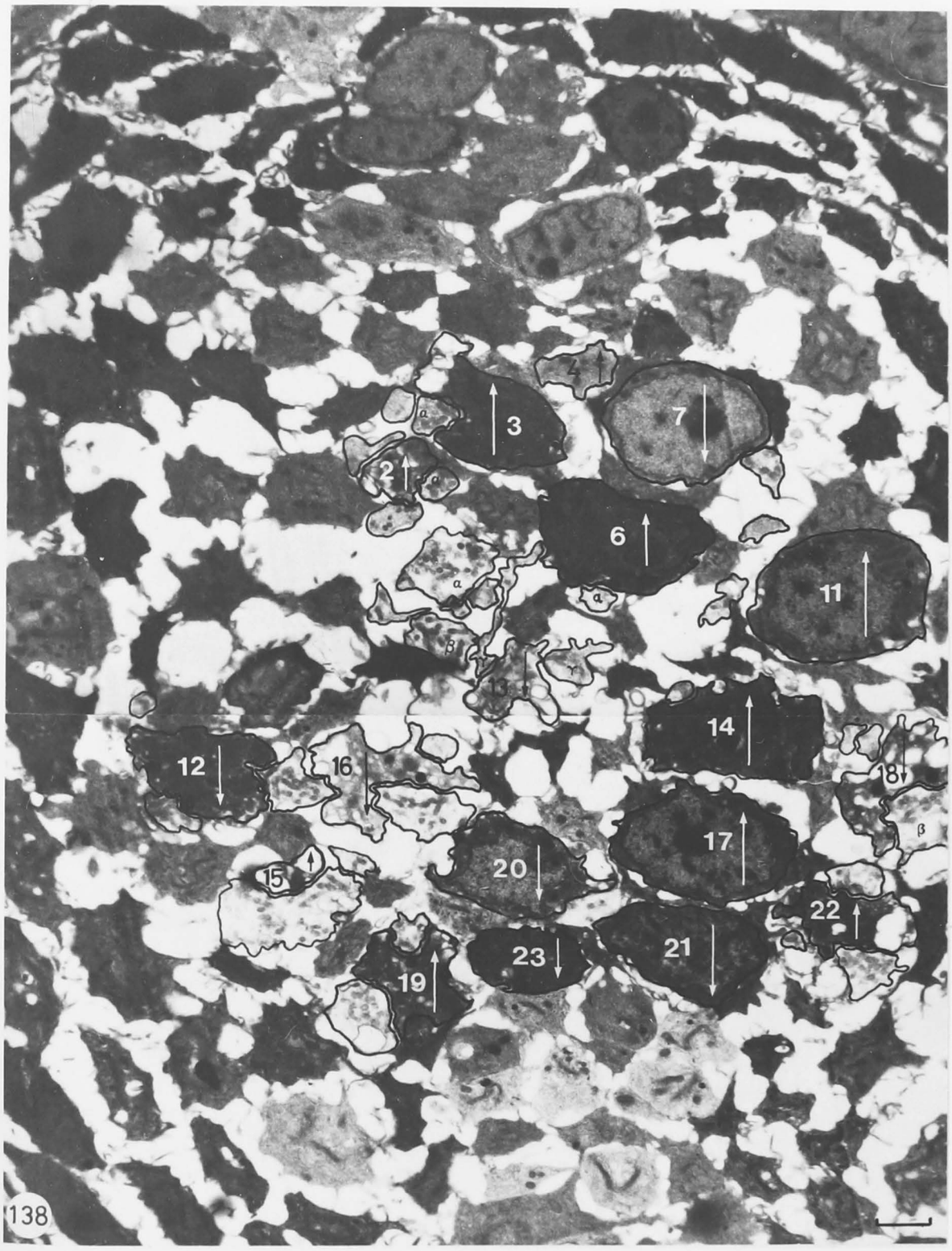

3
7
4
2
6
11
α
β
γ
13
14
12
16
18
17
20
15
22
23
21
19
138

Figure 57

Not all nerve fibres could be traced through from the basement lamella to the receptor cells. This is because the diameter of the fibres is very narrow in places and axons do not necessarily follow straight courses; some fibres literally disappear from one section to the next. The two fibres marked γ in the middle of the field show how small fibres can be.

Scale 1.0 μm.

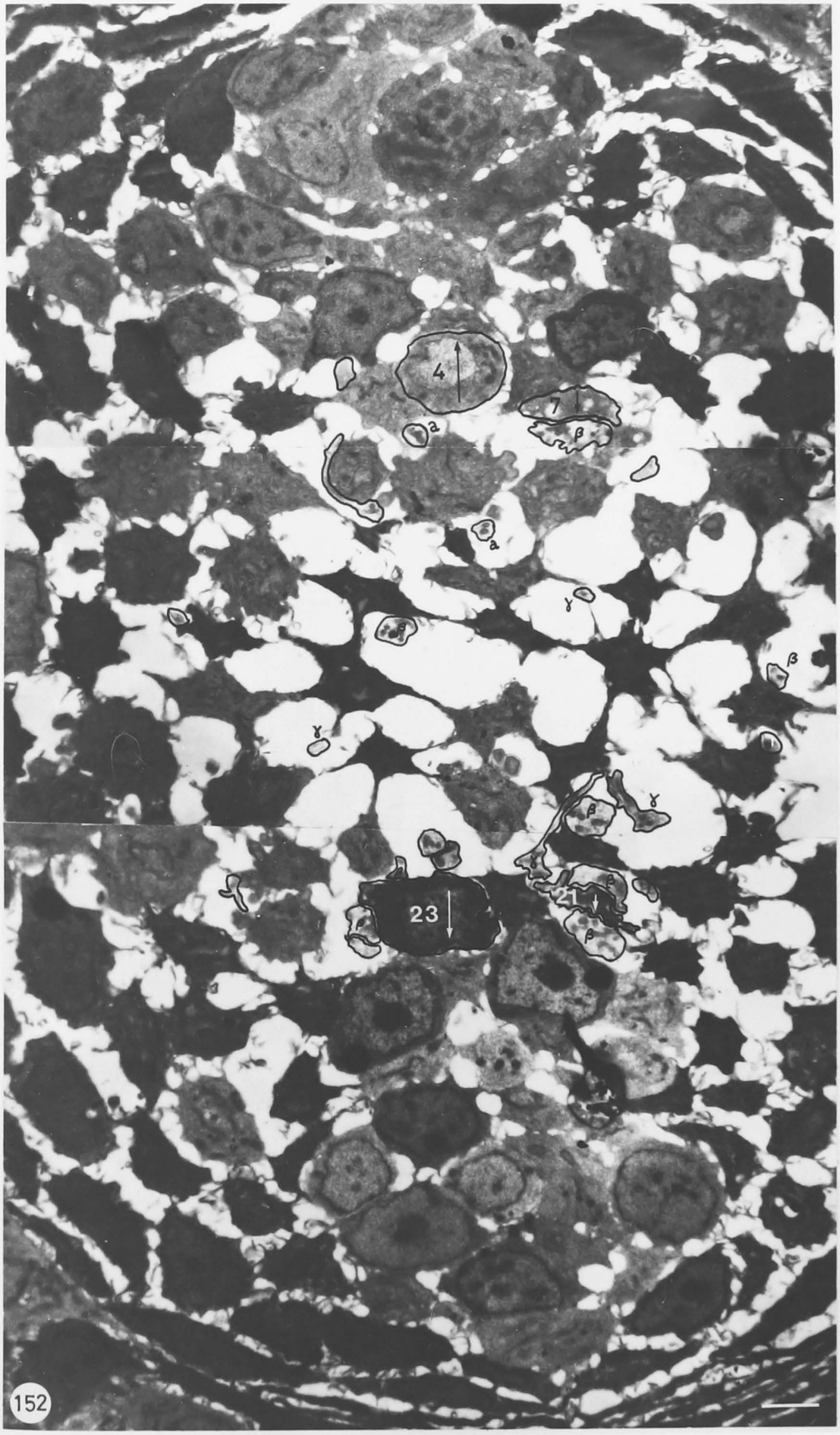

4
7
α
β
α
γ
β
γ
β
γ
23
21
β
β
152

Figure 58

This is a similar section to the previous one, the nuclei at the sides of the organ belong to so-called developing receptor cells. Scale 1.0 µm.

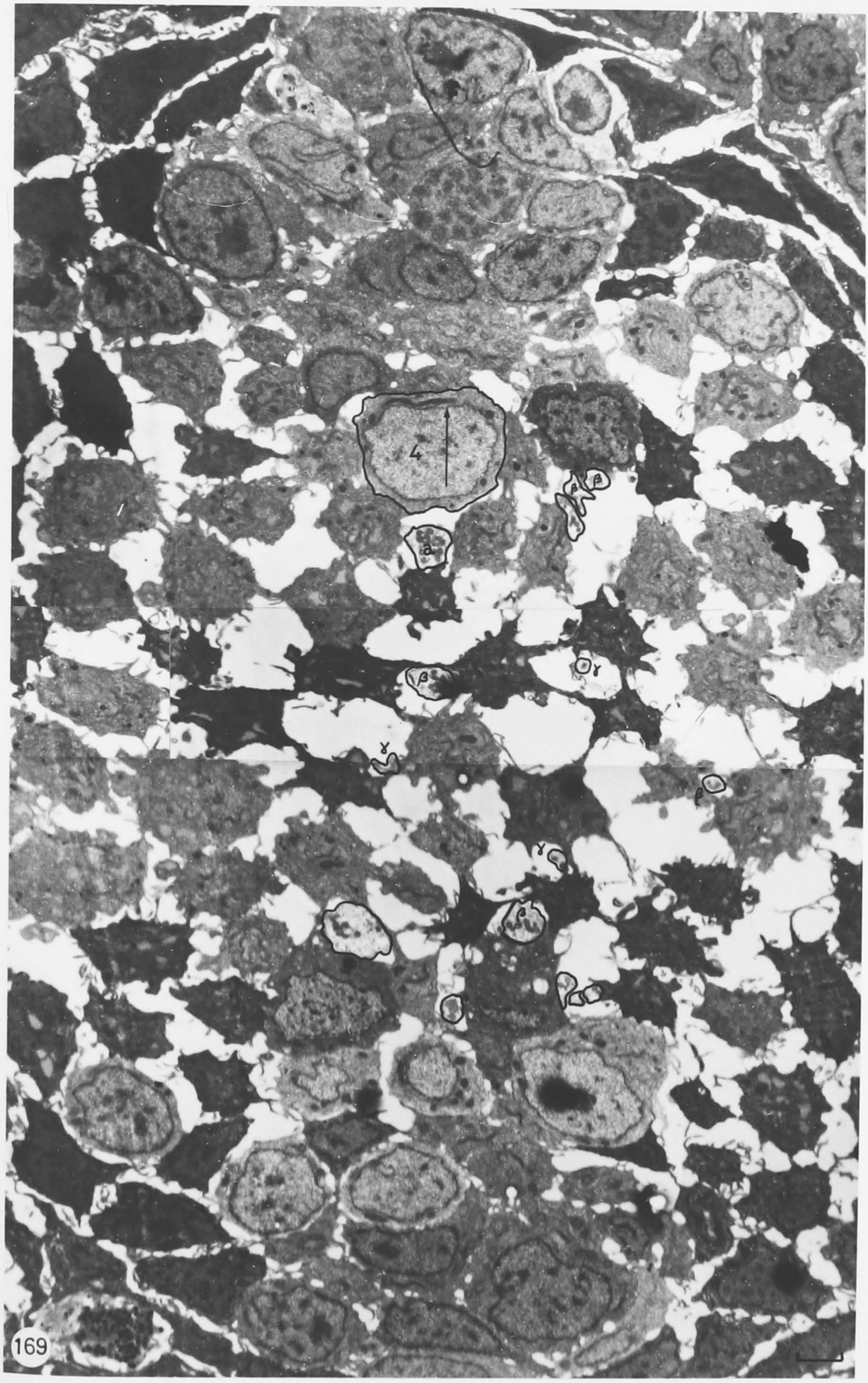
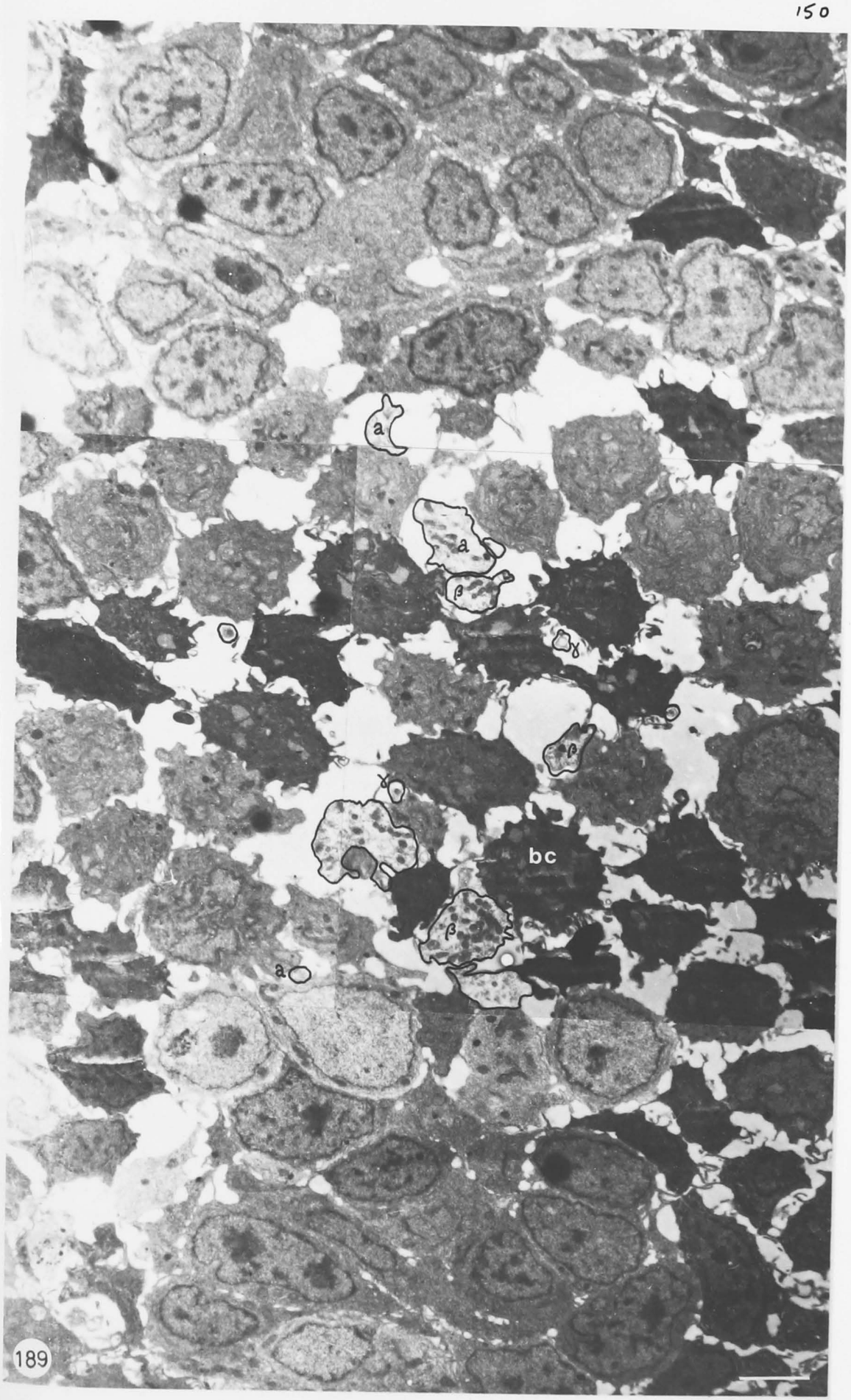

4
α
β
γ
β
γ
β
γ
169

Figure 59

This micrograph is from the region below the receptor cells but above the basement lamella. The basal cells (b.c.) have dense cytoplasm and enlarged cysternae of the endoplasmic reticulum. There are large intercellular spaces at this level.

Scale 1.0 μm.



189

Figure 60

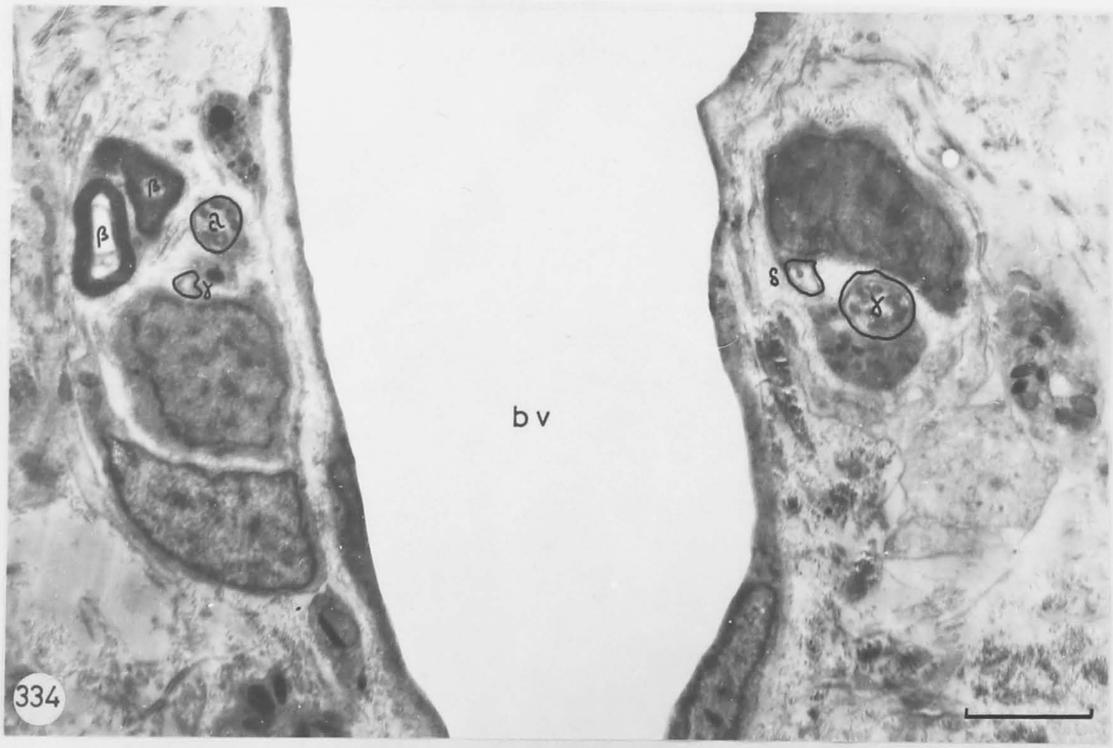
This micrograph from the series shows nine identifiable nerve fibres entering the base of the organ. Terminals of all but one class (δ) were identified at the receptor cell level. In this micrograph it is not possible to distinguish any other nerve fibres than those marked. The section is 60 to 70 μm below the cuticular plate region. b.l. = basement lamella. Scale 1.0 μm.


b.l
δ
α
β
γ
γ
β
α
280

Figure 61

This micrograph from the series shows the nerve fibres below the organ, running on either side of a blood vessel (b.v.). Myelination of nerve β is visible at this level.
Scale 1 μm.

Lower down myelination of fibres α and β is apparent but the nerves γ and δ are still unmyelinated. Other myelinated axons in the micrograph are associated with a different organ in the plaque.
Scale 1.0 μm.


β
α
γ
bv
δ
γ
334


γ
γ
δ
α
γ
γ
γ
β
361

Figure 62

This is the last micrograph in therseries showing that nerves α and β are of the large myelinated class and that nerves γ and δ are small myelinated fibres. The two unlabelled nerves innervate another organ in the plaque. It is not possible to distinguish unmyelinated axons in the bundle.

Scale 1.0 μm.


α
β
γ
δ
525

Figure 63

These four diagrams show internal (upper pair) and external views of a non-granulated ending. The ending was reconstructed in two halves to provide uninterrupted views of both internal and external detail. Synaptic bar distribution is clearly revealed and the sensory axon can be seen in the two right-hand diagrams. Although receptor cells never have more than one non-granulated terminal a single ending may enclose the bases of two adjacent cells. When this occurs both the cells have the same morphological polarization of the cilia at their apex.

Scale 2.5 μm.

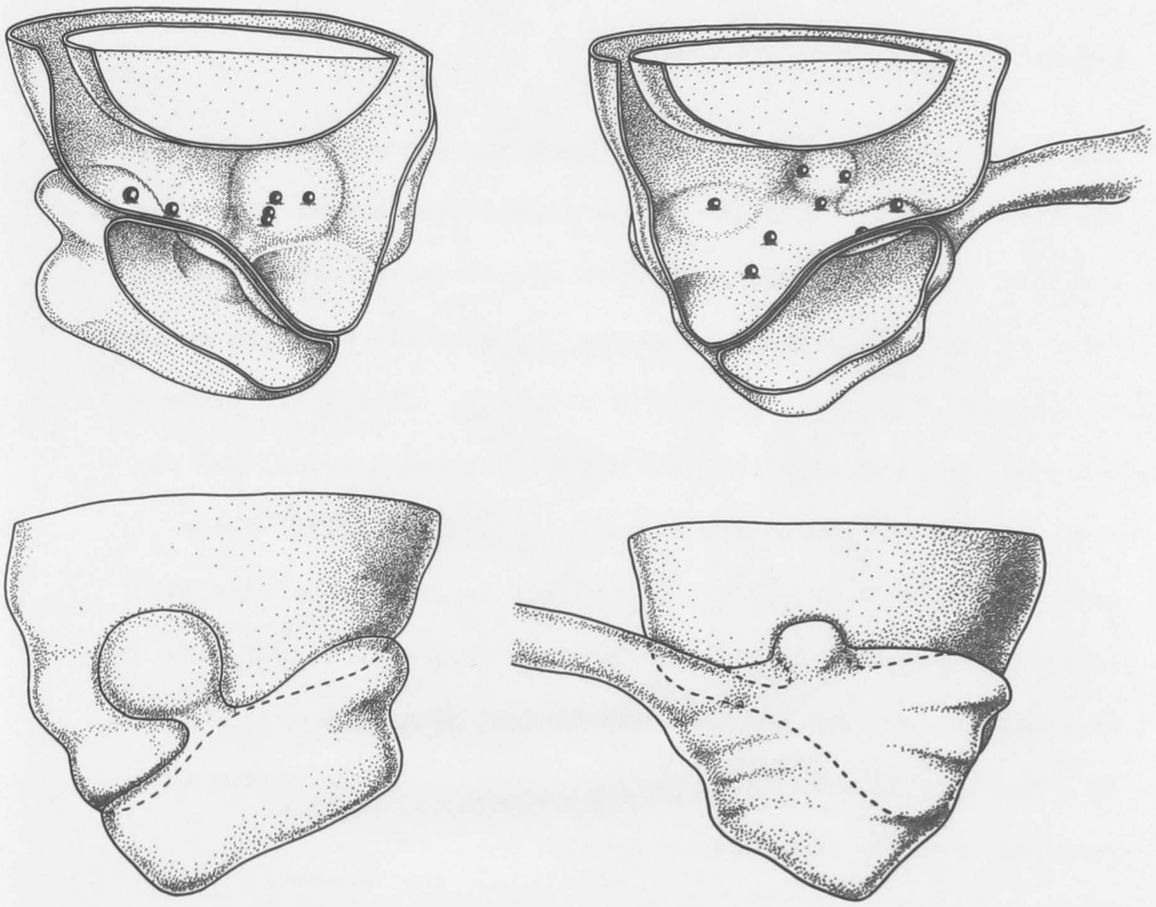


Figure 64

This diagram prepared from serial sections shows a reconstruction of the base of cell 13 and its nerves viewed from below. The base of the hair cell (h) is contacted by a single non-granulated nerve terminal (ng). A granulated ending (g) branches over the surface of the hair cell and its afferent terminal. This fibre makes synaptic contacts with both the non-granulated terminal and the hair cell at the two points 'x' and 'y' respectively. These synaptic contacts are of the *en passant* variety. The efferent fibre also branches to other hair cells at points 1 to 5, the destinations of branches were not traced. The micrographs shown in subsequent figures show selected detail from the series of sections used for the reconstruction.

Scale 0.5 μm.

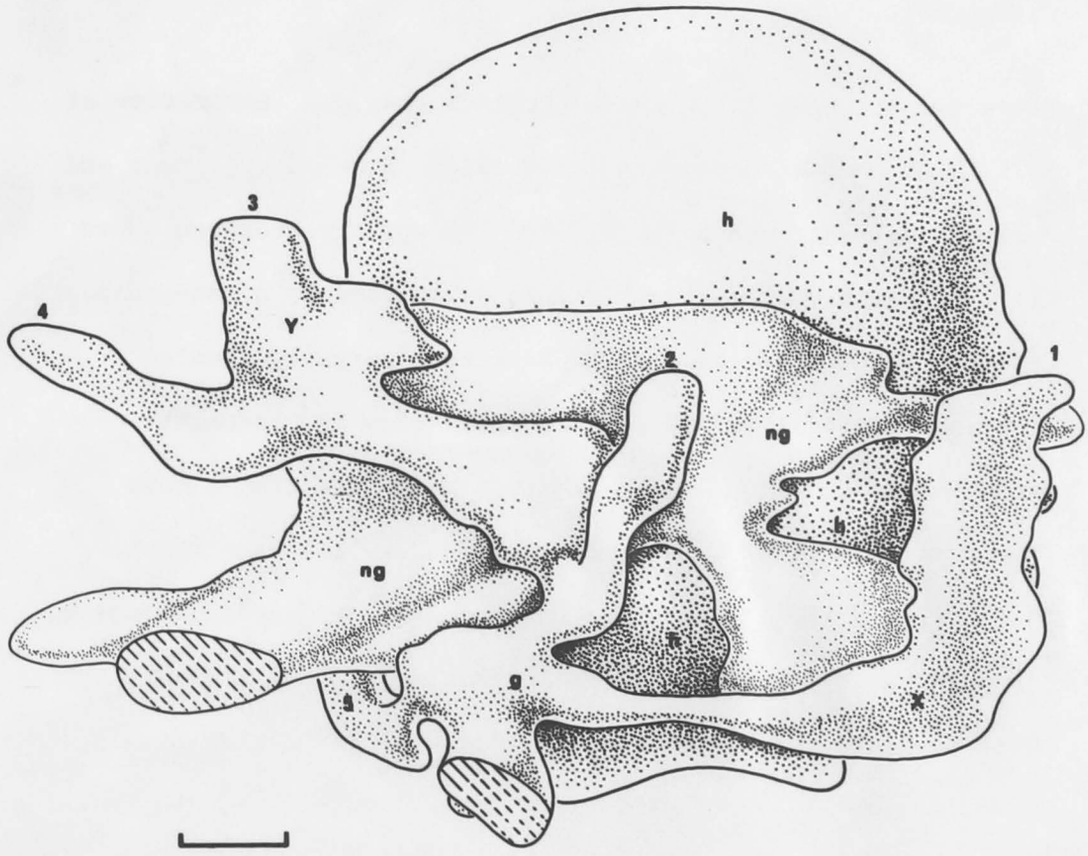

3
h
4
Y
1
Z
ng
h
ng
T
g
g
X

Figure 65

These two micrographs of thick sections show the innervation of cell 13 by granulated endings, revealing detail at points x and y of the reconstruction (Fig. 64). The upper photograph shows the lower part of the receptor cell surrounded by a non-granulated ending. This enidng was traced back to a large myelinated fibre designated β in the nerve bundle below the basement lamella of the organ. Two granulated endings marked α have been traced through to a small myelinated fibre. The right-hand of these two granulated endings forms a synaptic contact with the non-granulated terminal. The left-hand α fibre in the upper photograph terminates directly on cell 13 higher up. This contact is shown in the lower micrograph. Detail of both contacts is revealed at higher power in Figs 66 and 67 respectively. Scales 0.5 μm.

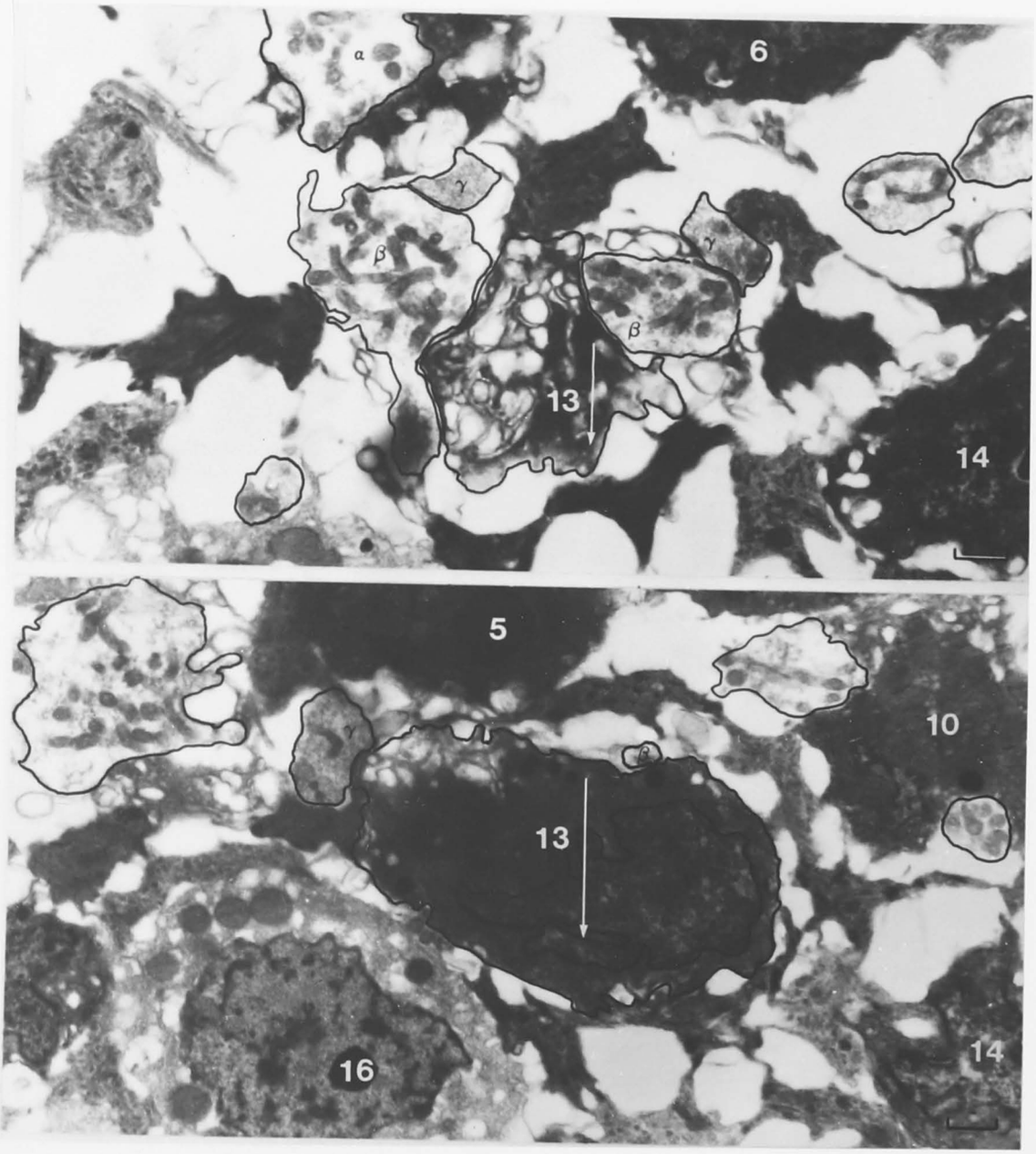

α
6
γ
β
γ
β
13
14
5
10
γ
13
16
14

Figure 66

This micrograph shows the presynaptic contact of the granulated ending (GN) upon the non-granulated terminal (NG) of receptor cell 13 at point x. The presence of the post-synaptic cistern in the non-granulated ending provides good anatomical evidence for synaptic contact. There is also good evidence for synaptic contact between the receptor cell and the non-granulated ending. Membrane thickening and the presence of a synaptic bar (sb) are criteria that have been used to indicate such functional contacts in conventional micrographs.

Scale 0.5 μm.

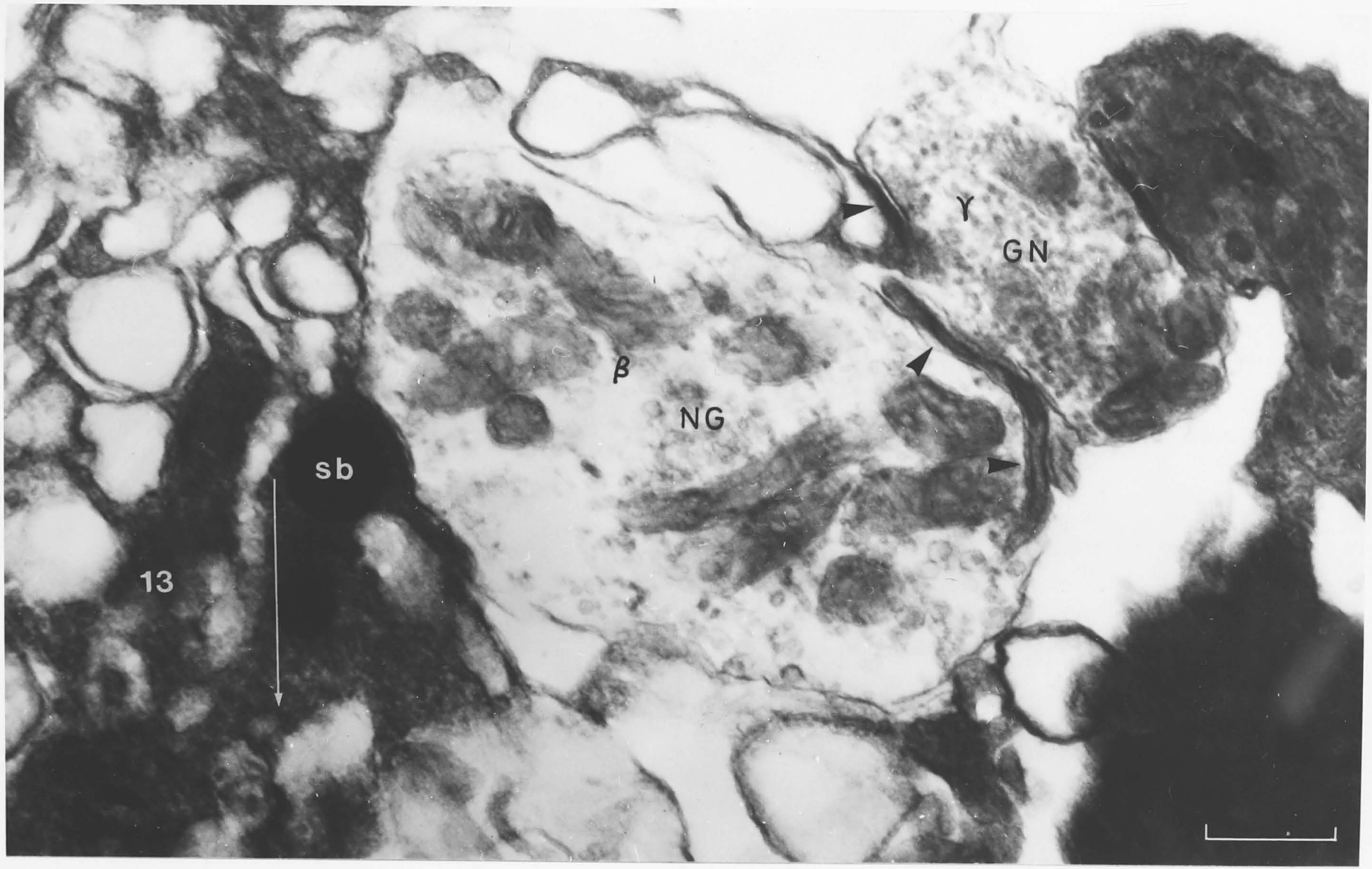

γ
GN
β
NG
sb
13

Figure 67

Detail of the contact of a granulated ending with receptor cell 13 at point y. In this case functional contact is directly onto the cell body. The granulated ending (g) contacts the receptor cell (r) on the right of the picture. A uniform synaptic cleft is visible and a post-synaptic cistern occurs in the receptor cell (arrow). Vesicles are concentrated near to the point of contact.

Scale 0.5 μm.

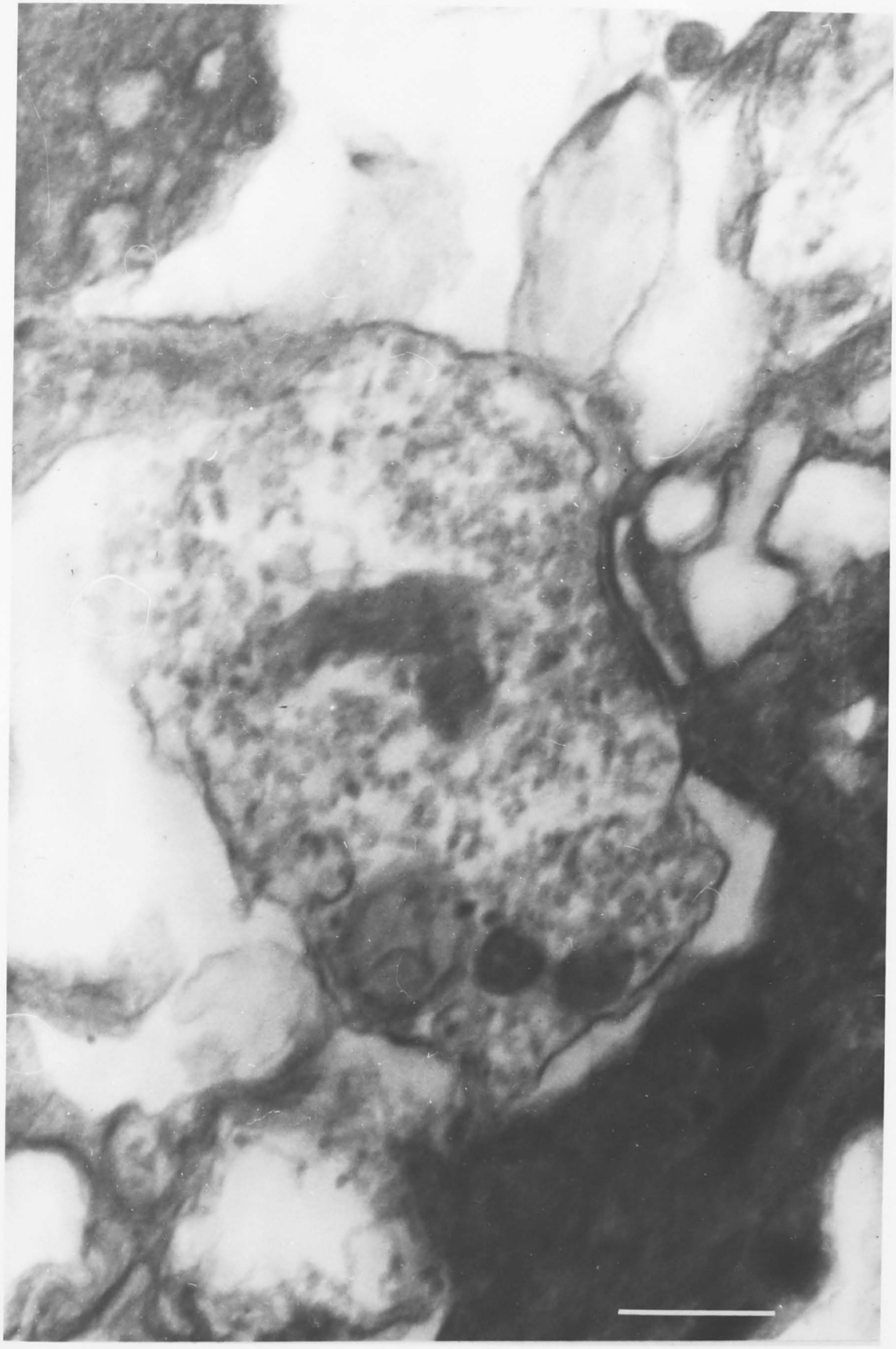


Figure 68

Selected sections from the series were used to reconstruct dense inclusions in cell 20. The number of each section is indicated by the figure in the lower left-hand corner of each photograph. 17 sections were used for the reconstruction.

Scale 1.0 μm.

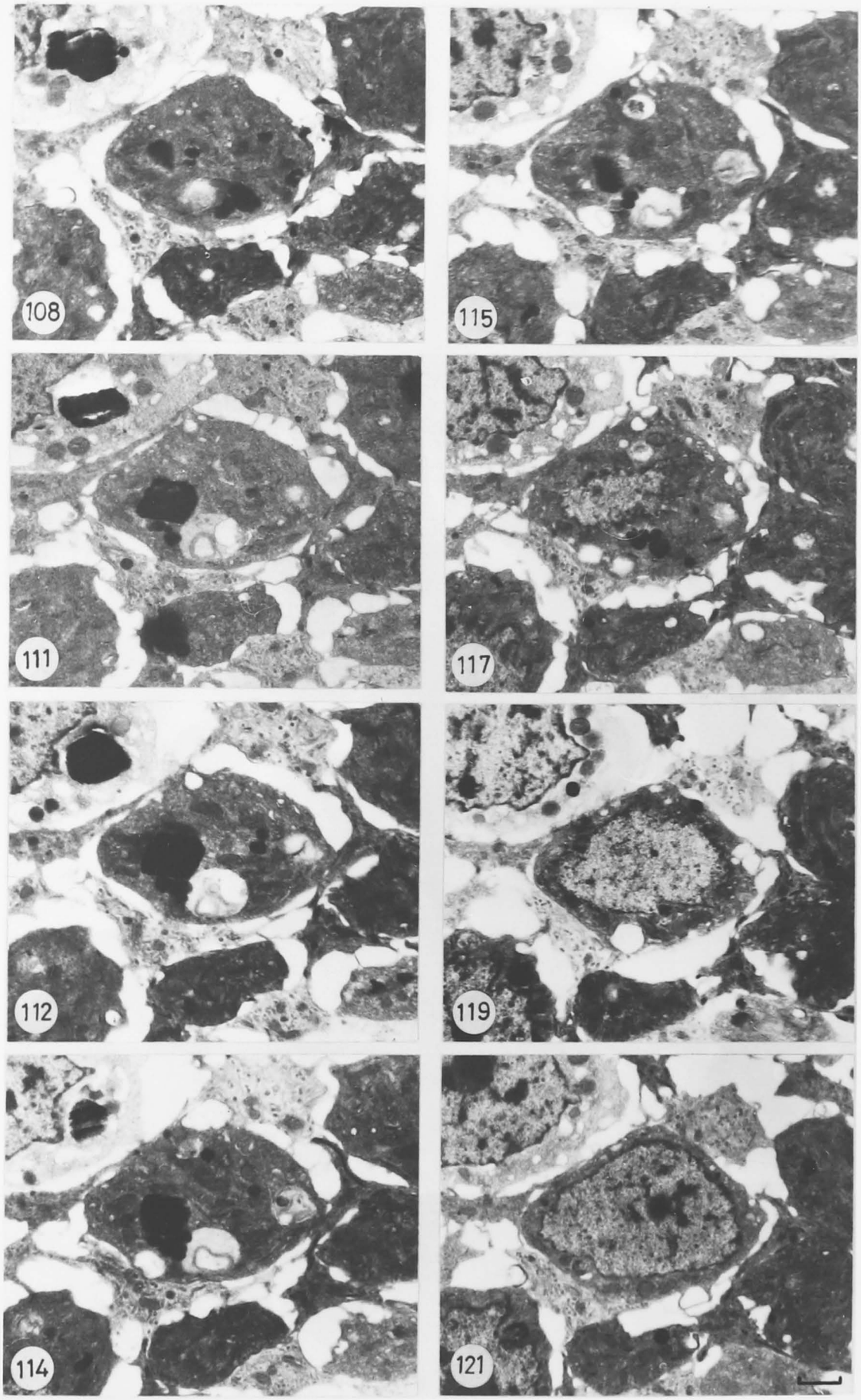

108
115
111
117
112
119
114
121

Figure 69

This illustration was reconstructed from serial 1/4 µm sections some of which are shown in Fig. 68. It shows details of dense inclusions just above the nucleus of cell 20.

Scale 1.0 µm.

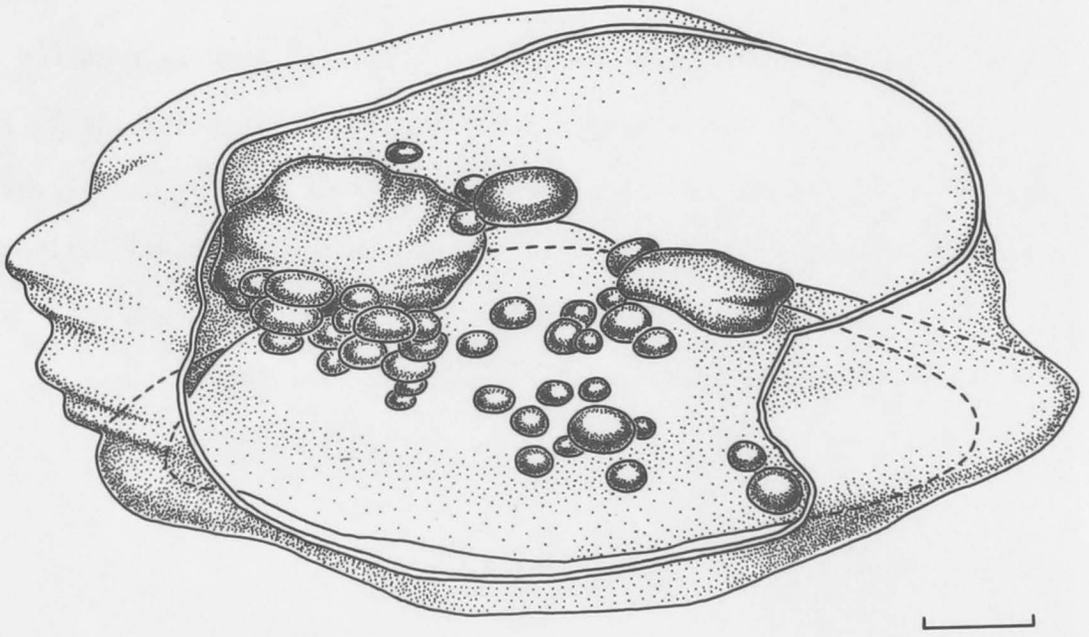


Figure 70

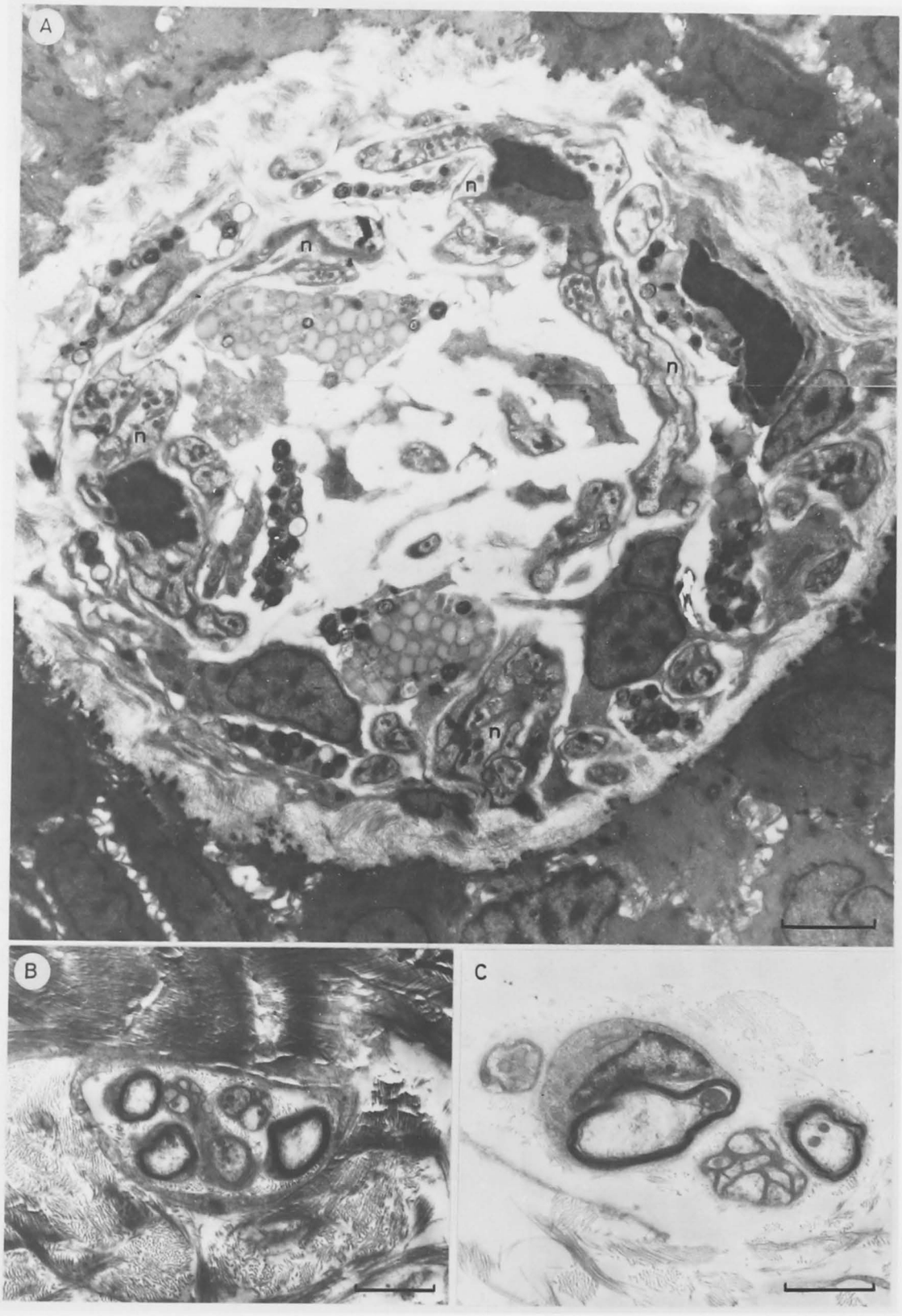
(A) This transverse section through the base of a tactile receptor reveals a rich innervation by unmyelinated nerve terminals (n.).

(B) and (C) These micrographs are cross-sections of nerve bundles entering two tactile receptors through the basement lamella. They consist of both myelinated and unmyelinated fibres and are completely separate from the lateral line nerve.

Scales A 2.0 μm

B 1.0 μm

C 1.0 μm


A
n
n
n
n
n
B
C

CHAPTER VIII

DISCUSSION

Most frogs and toads come out on land at metamorphosis and the lateral line system is completely lost (Larsell, 1934). *Xenopus* is unusual because the adult is totally aquatic and the lateral line is retained. The present investigation was designed to investigate the structure and function of the larval lateral line, to study the changes in the system at metamorphosis and to determine the relevance of these changes to a new mode of life. The combined techniques of electron microscopy, electrophysiology and behavioural analysis have been used in the investigation and it has been shown that much of the peripheral organization of the lateral line system is present in the tadpole. Physiological and behavioural experiments show that it is functional before metamorphosis but during metamorphosis there are changes in the anatomy and physiology of the lateral line which may affect the function of the system. These results are summarized at the ends of Chapters III to VII. In this discussion, the changes in the lateral line system at metamorphosis are considered in relation to the changing behaviour of *Xenopus* and in relation to the known changes in other systems of sense organs. Finally the details of hair cell innervation described in Chapter VIII are discussed in relation to the innervation of the receptor cells in the ear.

I. The lateral line system during development and metamorphosis

(i) Function in behaviour

The function of the lateral line in the tadpole is still unclear. It has been demonstrated that the system responds to differential stimulation but it does not appear to be essential for maintaining station in a water stream or for feeding behaviour. Thus tadpoles behave normally in a stream without visual cues after the lateral line nerves are cut. This agrees with the results of Dijkgraaf (1934, 1963) for minnows and Arnold (1969) for plaice which show that the lateral line is not involved in the rheotactic response. However, local stimulation of the lateral line with water jets elicits a turning response in the tadpole. A similar response in *Ambystoma* is associated with feeding behaviour (Scharrer, 1932; Copenhaver and Detwiler, 1940) and helps the animal to orientate towards a struggling prey. The response in *Xenopus* larvae is much less predictable and less accurate and it is difficult to assess its function because *Xenopus* is a filter feeder before metamorphosis. It is conceivable that the response is not important in normal behaviour but its presence indicates that larvae can detect differential stimulation of the lateral line system.

There is still the possibility that the larval lateral line responds to a stimulus parameter not examined here. While there is little doubt that the lateral line is primarily a

mechanoreceptor responding to local water disturbances (see Dijkgraaf, 1963), the spontaneous activity of the lateral line nerve is modified by temperature (Murray, 1956). However, careful behavioural experiments have shown that fish can distinguish between hot and cold water jets after the lateral line nerves are cut (Dijkgraaf, 1940, 1943). Recent electrophysiological experiments have shown that the system is sensitive to chemical stimuli (Katsuki and Hashimoto, 1969; Katsuki, Yanagisawa, Tester and Kendall, 1969; Katsuki, Hashimoto and Yanagisawa, 1970; Onada, Hashimoto and Katsuki, 1970). A chemosensory function might be important in the filter feeding tadpole for the location of water rich in organic material. In the adult, which is a scavenger as well as a carnivore this would also help it to locate decomposing food as well as struggling prey.

In fishes it is not yet resolved whether the lateral line is used in schooling behaviour. In general, it seems that chemical and visual clues are more important (Hemmings, 1963; Dijkgraaf, 1967) but Moulton (1960) showed that a blinded anchovy can join a school of normal fishes and follow sharp veering movements. It has been suggested that the near-field displacements caused by swimming fish provide the lateral line with information regarding the spatial separation of individuals within a shoal (Harris and Bergeijk, 1962). A primitive form of schooling behaviour occurs in *Xenopus* larvae. Within the confines of an aquarium, tadpoles tend to line up facing the same way and they also often swim on parallel courses across the tank. The continual motions of a tadpole's tail set up local water disturbances and another tadpole within the near

field would be able to detect these movements with the lateral line. In filter feeders such as *Xenopus* larvae there must be an optimum density of animals for maximum efficiency in collective feeding; if animals are too close, tadpoles refilter water already used by their neighbours. The lateral line may be used in regulating the distance between tadpoles in the shoal. It could also be used to detect the movements of predators although the turning response towards the source of a stimulus can have little protective value. In fish the swimming dynamics of different species produce unique patterns of water displacement which might provide a basis for species recognition (Harris and Bergeijk, 1962).

After metamorphosis, there is little doubt that the lateral line is used in feeding behaviour (Dijkgraaf, 1963, 1967). Unfed adults always respond to local water displacements near the head. The response is very characteristic and the toad turns towards the stimulus and sweeps the area in front of the mouth with the forelimbs. Water disturbances can be detected from considerable distances especially in shallow water. Thus the adult response is much more refined, more predictable and requires greater motor co-ordination than the turning behaviour of the tadpole. The larval response uses the tail and therefore involves a completely different set of neuromuscular connections, so it seems unlikely that it is merely a stage in the development of the adult behaviour.

(ii) Morphological and physiological changes during development

From the examination of the fine structure of larval organs it is apparent that the two classes of receptor cell found in

adults organs (Dijkgraaf, 1963; Görner, 1961, 1963; Harris and Milne, 1966) are present in young larvae. The two types of receptor cell have their hair cells morphologically polarized in opposite directions and the plane of polarization is consistent with a directional sensitivity to displacement of the hair process, maximal at right angles to the long axis of the plaque.

There are two types of nerve ending in contact with each receptor cell, which are thought to have afferent and efferent functions respectively. A dual innervation of hair cells originally described in the inner ear of vertebrates (Engström, 1958), occurs in the lateral line organs of adult *Xenopus* (Delaveuve and Szabo, 1966) and has also been described in many other acoustico-lateralis organs (Smith, 1961; Trujillo-Cenóz, 1961; Kimura and Wersäll, 1962; Flock, 1965; Hama, 1965; Lindeman, 1969; Spoendlin, 1969; and others). Evidence for an efferent function of granulated endings has come from degeneration studies (Engström and Fernández, 1961; Iurato, 1962; Spoendlin and Gacek, 1963; Smith and Rasmussen, 1963; Hillman, 1969a; and others) and it is therefore deduced that the non-granulated endings are afferent. Various other types of ending have been described in the sensory epithelium of the inner ear (Gacek, 1960; Spoendlin and Lichtensteiger, 1966; Lindeman, 1969, and others) and one type of process observed in the larval lateral line could represent the Ne 3 type of ending found in the sensory epithelium of the labyrinth in the thornback ray (Lowenstein, Osborne and Wersäll, 1964). However, there is no evidence that this structure is of neural origin nor that it makes functional contact with the hair cells.

From the electrophysiological experiments described in Chapter IV it is concluded that the lateral line system is functional by stage 54. At this stage the plaques are directionally sensitive to water currents with a response proportional to the logarithm of the current velocity. Experiments revealed no difference between the responses of larval organs and the responses of adult organs described by other authors (Görner, 1961, 1963; Harris and Milne, 1966). Directional sensitivity is consistent with the morphological polarization of hair processes. The only change in sensory innervation at metamorphosis is the diameter of the fibres which increases during development with a corresponding increase in the conduction velocity of afferent units.

On the other hand there are significant changes in the efferent system. Unmyelinated efferent fibres in the tadpole become myelinated at metamorphosis. In the developing limb nerves of rat foetuses myelination is essential before co-ordinated movements can appear (Peters and Muir, 1959). The lack of myelination of the lateral line efferent fibres in the larva may therefore reflect an unspecialized and immature function. There are also changes in the patterned activity of efferent units. In the tadpole they fire only in association with body movements, being active in regular short bursts during periodic gill movements and continuously during tail flexions. As locomotion becomes increasingly dependant upon the hind-limbs efferent activity becomes synchronous with motor activity in the sciatic nerve instead of with tail movements and the periodic activity associated with larval respiration disappears. The

persistence of efferent activity after curarization in both tadpoles and adults suggests that the firing pattern is not dependant upon sensory feedback but is generated centrally in parallel with motor activity in the neuromuscular system. The new patterns of efferent activity are presumably associated with the readjustments of intracentral connections necessary for the changing methods of locomotion and respiration.

In adult *Xenopus* (Görner, 1967) and fish (Hashimoto, Katsuki and Yanagisawa, 1970), efferent activity can also be elicited by ipsilateral and contralateral stimulation of the lateral line system. Efferent units are known to inhibit afferent activity in the adult lateral line nerve (Russell, 1968; Hashimoto, Katsuki and Yanagisawa, 1970) and it is thought that the efferents may provide a system of lateral inhibition within the lateral line. This would improve stimulus localization and would be especially useful in an animal depending upon its lateral line for the capture of prey. Lateral inhibition appears to be absent in the tadpole and this may explain the poor localization of stimuli shown in behavioural experiments on the larva. Although inhibition of afferent activity has not been shown in the tadpole, it is likely that the function of efferent fibres is to prevent overstimulation of the lateral line by the tadpole's own movements. It is interesting that vibration sensitive crustacean interneurons are inhibited during activity of the walking legs (Taylor, 1968). It may similarly be significant that turning responses could not be elicited in swimming tadpoles.

(iii) Positional changes in plaque distribution at metamorphosis

The changes in gross morphology which occur at metamorphosis (see Fig. 1) result in positional changes in the distribution of lateral line plaques. Since morphological and physiological evidence indicates that organs are directionally sensitive before these changes occur, it follows that the input from any organ signals events in different parts of the environment at different stages in development. A similar situation exists in the visual system of *Xenopus* (Gaze, 1970). Prior to metamorphosis vision is monocular and the retino-tectal connections are entirely contralateral. As binocular vision develops with the repositioning of the eyes, it is accompanied by the formation of an ipsilateral projection linking corresponding points in the projected fields of the two eyes. As there are changes in the position of lateral line organs at metamorphosis it is necessary to postulate similar developments of central connections if the system is to be used for accurate localization.

The functional consequences of the repositioning of plaques is well demonstrated in the supra-orbital row. At metamorphosis the plaques of the supra-orbital and infra-orbital rows become regrouped radially around the orbit of the eye so that their axes of maximum sensitivity are divergent. This allows a much more precise localization of stimuli than the arrangement in the tadpole where the supra-orbital plaques are in a row facing the same direction and with little angular separation of the maxima of sensitivity curves.

When *Xenopus* is at the surface to breathe the plaques on the dorsal part of the head, including the supra-orbital plaques, are in an ideal position for the detection of surface waves. A small disturbance on the surface creates waves which are propagated over the surface but which are rapidly damped out with depth (for mathematical proof of this assumption, see Appendix II). The further an animal is from a wave source on the surface the nearer to the surface it must be in order to detect the stimulus. Good behavioural evidence for this comes from the anatomy and behaviour of surface feeding fishes (Schwartz, 1967, 1970). In *Fundulus notatus*, turning responses to waves from a water drop on the surface are only obtained when the fish is actually in contact with the surface. Other species are able to respond to nearby surface disturbances at depths of several centimetres but only respond to distant stimuli when they are at the surface (Schwartz and Hasler, 1966). Thus surface waves arising at a distance would stimulate mainly the head organs of a surfacing toad and stimulus localization will be largely dependant upon these organs because other lines are much deeper in the water. There can be little comparison of stimuli between the deeper lines and therefore the organs on the head must localize a stimulus more accurately than is normally required of a small group of organs. The radial array of lateral line plaques around the eye potentially provides better stimulus localization within the plaques of the supra-orbital row because of the increased divergence of their axes of maximum sensitivity.

II. Sense organs at metamorphosis

Sense organs may be modified in one of three ways at metamorphosis. A particular sense organ system may be functional during only one part of the life cycle; the larval system may be a functional stage in the development of the adult with refinements at metamorphosis suiting it for the exacting requirements of more complex adult behaviour; or it may be functional at both stages but specially adapted for different roles in the larva and the adult. The changes may involve both the reorganization of the peripheral sense organs and the establishment of new central connections.

Jacobson (1970) suggests that plasticity of connections within the nervous system is the limiting factor governing the development of behaviour. He argues that there are two extreme classes of neuron in morphogenesis: the first, mainly afferent and efferent neurons with long axons, are rigidly specified early in development; while the second class, which consists of interneurons with short axons, has 'lax specification'. New cells of this type are generated until late in ontogeny. Most neurons would be intermediate between the two extremes and those with least specification would be most responsible for the plasticity of the developing nervous system. Sense organs whose significance in behaviour is modified at metamorphosis, and those in which there are important anatomical changes may require some changes in their central connections, and are probably associated with central interneurons of the type 2 class. Sensory connections which are established early in life and maintain their function in the adult fall into Jacobson's type 1 class.

From the recent review by Gaze (1970) it seems that most adult sense organ systems fall into the category of refined versions of an already functional larval system. A good example is the amphibian visual system. The major connections with the brain are established in the larva (Sperry, 1963) and they are physiologically patent before metamorphosis (Gaze, 1970). The changes which occur in the eye at metamorphosis are mainly small adjustments such as the expansion of the dendritic fields of ganglion cells (Pomeranz and Chung, 1970). The major change of functional importance is the development of binocular vision with the establishment of new intracentral connections to combine the inputs from left and right eyes.

In other amphibian systems, such as the innervation of the skin and the eye blink reflex, the experimental evidence indicates not only central but also peripheral changes. Reversed scratch reflex experiments (Miner, 1956; Jacobson and Baker, 1968, 1969; Baker and Jacobson, 1970) show that point to point discrimination of tactile stimuli only appears some time after metamorphosis (Gaze, 1970). Immediately after metamorphosis the response to tactile stimulation of skin transplanted from belly to back in the tadpole, produces a leg movement which is directed to the transplanted skin although it is in an inappropriate position. Later, stimulation of the transplanted skin elicits a reflex directed at the skin's normal location. As the transplanted skin is innervated by the local normal cutaneous innervation of the area it was suggested that the misdirected reflex occurs because the central connections of these nerves must be modulated according to the origin of the transplanted skin.

The normal development of the amphibian eye blink reflex is generally interpreted in terms of the modulation of central connections (Weiss, 1942). Adult newts close the eye in response to tactile stimulation of the skin. This behaviour appears progressively at metamorphosis. Initially only strong stimulation of the centre of the cornea can elicit eyelid closure. As development proceeds, the threshold decreases and the reflexogenic area expands to include the area from the ear to the nose. Shortly after metamorphosis the reflexogenic area contracts again until the response is elicited only by stimulation in the immediate vicinity of the eye. Tactile stimuli on the nose which formerly elicited the blink now produce reflex depression of the head (Sperry and Miner, 1949). Although the narrowing and differentiation of the reflexogenic zones must involve central changes, it has not been excluded that there are also changes in the sensory endings in the different areas of skin involved. Modulation implies that central connections are influenced by input from the periphery. The type of reorganization necessary for the gradual appearance of the misdirected scratch reflex could be due to the formation of new sense organs in an expanding area of skin (Gaze, 1970), while the decreasing reflexogenic area of the eye blink could be attributed to the death of inappropriately connected neurons.

An example where there is a major anatomical reorganization together with a radical change in function at metamorphosis is the anuran acoustic apparatus. According to one theory, the ear in larval *Xenopus* is specialized for a very unusual role (Bergeijk, 1959). In aquatic vertebrates whose bodies are almost the same density as the surrounding medium, a prerequisite for sound

perception is the presence of a gas-filled chamber. Mechanical coupling between this chamber and the sensory hairs of the inner ear transforms sound into meaningful sequences of activity in the auditory nerve. In anuran tadpoles the developing lungs may fulfil this function as they are the only air filled sacs and they are mechanically coupled to the round window of the larval ear (Witschi, 1949, 1950, 1955; Bergeijk and Witschi, 1957). However, recent experiments have shown that the lungs in *Xenopus* tadpoles are poorly suited for auditory perception because they have the properties of a mechanical resonator with low damping (Bergeijk, 1959). Not only does such a chamber 'ring' at its resonant frequency after impulsive excitation but the spectrum of ambient sounds is considerably distorted. Continuous and remarkably regular spontaneous microphonic potentials recorded from the saccule are thought to be caused by the movements of the resonating lung. It is suggested that the lungs are more important in the detection of static pressure changes which modulate the resonant frequency and so provide a system for depth discrimination (Bergeijk, 1959). Periodic excitation from the heartbeat causes the lung to ring at a frequency which changes with depth as the volume of the lung alters.

At metamorphosis mechanical connection between the lung and the round window is lost and sound is transmitted to the ear via the oval window. In the adult the saccule is stimulated by vibrations (Ashcroft and Hallpike, 1934) and is sound sensitive (Glekin and Erdman, 1960). There is convincing evidence that the saccule in fish is also sensitive to vibrations (see e.g. Lowenstein and Roberts, 1951). Thus it seems that the acoustic apparatus is

adapted for different roles before and after metamorphosis, although at both stages the stimulus is transduced by shearing forces acting on the hair processes of the saccular epithelium. Nevertheless, modifications of central connections must occur because of the changing role of the system in behaviour.

The lateral line in *Xenopus* has features of both the second and third categories of sense organ development. While it is obviously a stage in the development of the adult system, the loss of many sense organs and the redistribution of others at metamorphosis argues against it being only functionally significant to the adult. The afferent nerves of the lateral line are formed early in development and can be classified as Jacobson's type 1 neurons but the changing patterns of efferent activity suggest that there are central adjustments attributable to type 2 neurons. The myelination of the efferent fibres at metamorphosis reflects the refinement of the system during development. The redistribution of organs are probably accompanied by alterations in central connections if the lateral line is used for localization in both larval and adult stages. The reorganization of organs on top of the head is undoubtedly a specialization for adult behaviour.

Although studies on developing sensory systems show that the establishment of new central connections is necessary, very little is known of the levels at which this takes place and nothing is known of the mechanisms involved. The best demonstration of new connections comes from studies on the development of the ipsilateral projection of the eye in the frog (Jacobson, 1971) and *Xenopus* (Gaze, Keating, Székely and Beazley, 1970) but even here, little

is known of the actual pathways concerned (see Gaze, 1970). Undoubtedly cell proliferation and death as described by Hughes (1968a) must play a major role but as yet our evidence is on the gross scale and there are no single cell dynamic studies, or investigations of small populations of neurons, during development and behavioural studies. Jacobson's (1970) hypothesis of the differential specificity of developing neurons provides a useful framework for identifying the changes which are taking place but contributes little to the understanding of the mechanisms underlying the changes.

III. Innervation of hair cells in the lateral line and the efferent system of the ear

By tracing nerve fibres in serial EM sections, it has now been shown that the non-granulated endings are the terminals of afferent fibres and the granulated endings are the terminals of small myelinated efferent fibres. Each receptor cell is innervated by a single non-granulated ending although one ending may sometimes be shared by two adjacent cells. The synaptic sites between the hair cells, as determined by synaptic bar distribution, vary in number and position from one receptor cell to the next.

The granulated endings make synaptic contacts with both the hair cells and the afferent terminals. It is presumably at this level that efferent units inhibit afferent activity. The function of this dual inhibition of a single hair cell is difficult to explain. However, it is possible that by inhibiting both the hair cell and its afferent fibre simultaneously a more efficient inhibition is achieved as on-going afferent activity will be

suppressed as well as receptor cell transduction. Presynaptic inhibition would also prevent activity in the afferent nerve arising from residual transmitter substance.

There is considerable evidence that the inner ear is evolved from a primitive lateral line (Pumphrey, 1950; Bergeijk, 1967). The arrangement of nerve terminals on the vestibular and acoustic hair cells varies according to the type of hair cell and its location within the organ but all have some of the features found in the synaptic organization of the lateral line organ (Fig. 71). Two types of hair cell are found in the labyrinth, one has presynaptic efferent endings, the other has efferent endings contacting the hair cell directly. In the organ of Corti both direct and presynaptic inhibitory contacts are found on the inner hair cells while the outer hair cells have only direct contact with efferent terminals.

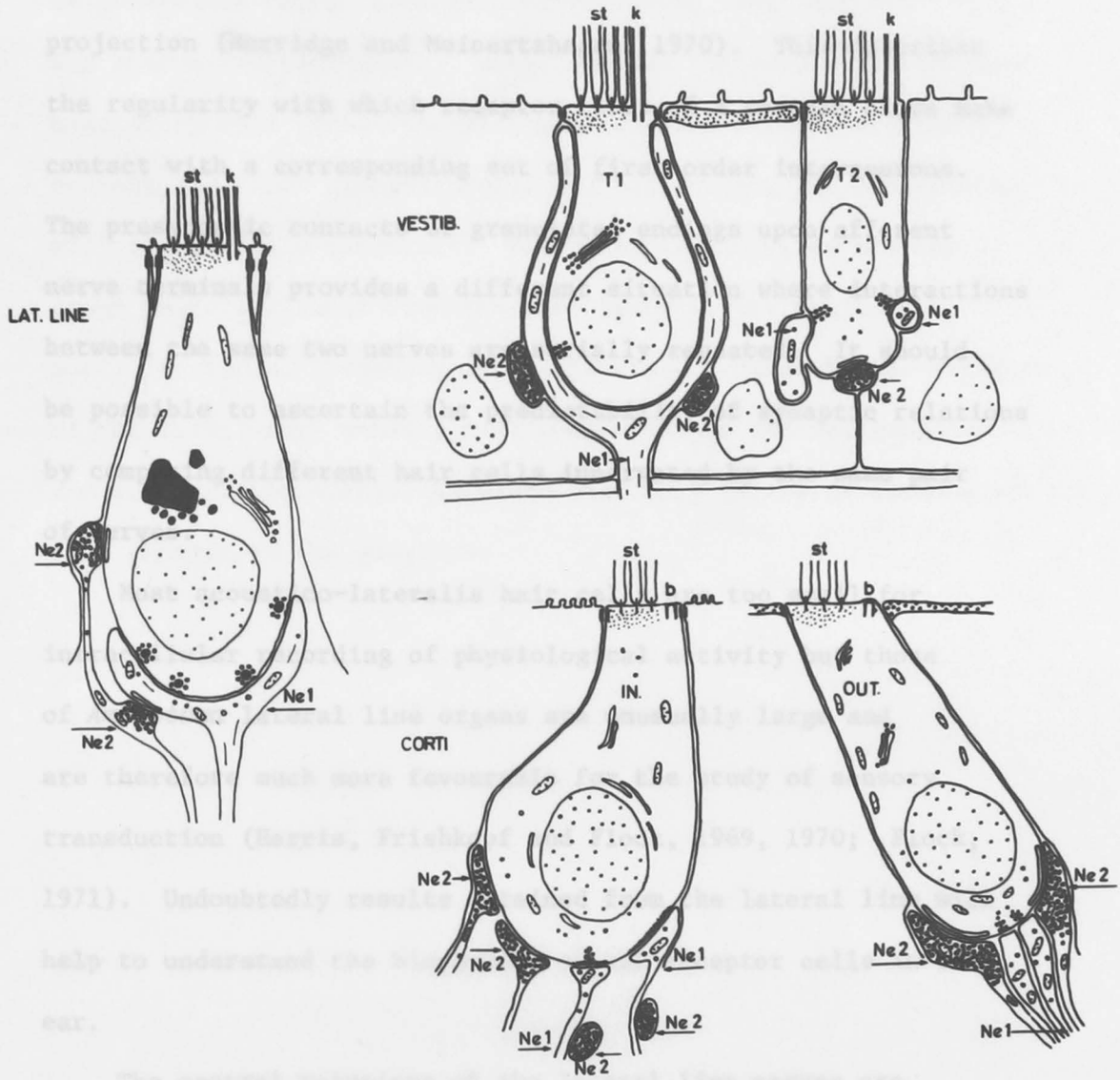
Unmyelinated fibres are present in all acoustico-lateralis nerves but their destinations and functions are unknown. In the labyrinth they enter the sensory epithelium and pass between the supporting cells but their further course is unclear (Lindeman, 1969). In addition there is a system of adrenergic fibres terminating outside the labyrinthine epithelium (Spoendlin and Lichtensteiger, 1966) and an intraepithelial nerve plexus which persists after section of efferent nerve tracts (see Lindeman, 1969). The function and synaptic relations of these systems will be clarified using serial section studies together with careful reconstruction work. Perfection of histochemical methods and staining techniques for electron microscopy should improve interpretation of anatomical studies.

Figure 71

This figure summarises the patterns of connections between the hair cells and their innervations in the lateral line system and the mammalian inner ear. For conformity the terminology of Engström (1958) has been used for the different classes of nerve terminal. Thus afferent or non-granulated endings are labelled Ne 1 and efferent or granulated endings are labelled Ne 2. The primitive situation is presumably that of the lateral line system. Here each hair cell has a single afferent terminal at its base with numerous synaptic contacts between the hair cell and the nerve ending. Efferent terminals have functional contact with the afferent terminal and also make direct contact with the hair cell itself.

In the labyrinth there are two types of hair cell designated type 1 and type 2. The type 1 hair cells have direct contact with the afferent ending only, this forms a chalice almost completely surrounding the cell. Efferent endings terminate upon this chalice and not on the hair cells. The type 2 hair cells are contacted directly by endings of both classes but there is apparently no synaptic contact between the afferent and efferent nerves.

In the organ of Corti two types of hair cell are present, the outer and inner hair cells. Both types of ending contact the outer hair cells directly with few direct contacts between efferent and afferent fibres. This is not the case with the inner hair cells where there are many efferent terminals ending upon afferent fibres. The diagrams of the inner ear hair cells are modified after Wersäll (1968).


st
k
LAT. LINE
Ne2
Ne1
Ne2
st
k
T1
VESTIB.
Ne2
Ne2
Ne1
st
k
T2
Ne1
Ne1
Ne 2
st
b
IN.
CORTI
Ne2
Ne 2
Ne1
Ne1
Ne 2
Ne 2
st
b
OUT.
Ne 2
Ne 2
Ne1

IV. Outlook

One way of studying the specificity of nerve cells is to ascertain the predictability of connections within an ordered projection (Horridge and Meinertzhagen, 1970). This describes the regularity with which receptor cells of a certain class make contact with a corresponding set of first order interneurons. The presynaptic contacts of granulated endings upon afferent nerve terminals provides a different situation where interactions between the same two nerves are serially repeated. It should be possible to ascertain the predictability of synaptic relations by comparing different hair cells innervated by the same pair of nerves.

Most acoustico-lateralis hair cells are too small for intracellular recording of physiological activity but those of *Ambystoma* lateral line organs are unusually large and are therefore much more favourable for the study of sensory transduction (Harris, Frishkopf and Flock, 1969, 1970; Flock, 1971). Undoubtedly results obtained from the lateral line will help to understand the biophysics of the receptor cells in the ear.

The central relations of the lateral line nerves are particularly challenging. In systems which depend upon the spatial localization of environmental stimuli it is usual to find an ordered central projection of sensory nerves within the brain (Jacobson, 1970) but this order is not apparent in the central connections of the lateral line nerves. In the medulla each first order ending from the lateral line divides

into ascending and descending branches which contact many second order neurons. The second order cells receive additional inputs from other cranial nerve roots (Herrick, 1948). There must be order in the projection but its exact nature can only be determined using intracellular recording, cell marking and anatomical techniques. Golgi studies combined with electron microscopy will reveal the different classes of second order neurons and their synaptic relations but tracing the projection will be extremely difficult. The distances involved are too great to follow axons from the end-organs to the brain in serial sections. Degeneration studies are limited by the location of the first order sensory cell body within a cranial ganglion and degeneration within the brain will only follow if lesions are made central to the ganglion.

APPENDIX I

I. Fixation of tissue for electron microscopy

Tissue was fixed using glutaraldehyde and osmium tetroxide according to the methods recommended by Pease (1964).

(i) Glutaraldehyde fixative after Sabatini, Bensch and Barrnett (1963; Sabatini, Miller and Barrnett, 1964)

Solutions

(a) $NaH_2PO_4.2H_2O$ 11.86 gm in 1000 ml distilled water

(b) $Na_2HPO_4.2H_2O$ 57.67 gm in 1000 ml distilled water

Preparation of fixative

10 ml (a)

10 ml (b)

94 ml distilled water

10 ml 25 per cent glutaraldehyde

The fixative has a pH of approximately 7.5 and tissue is fixed for two hours at 4.0°C. The first three constituents mixed in the proportions given, provide a rinse to wash off excess glutaraldehyde prior to post-fixation.

(ii) Osmium tetroxide fixative after Palade (1952)

Solutions

(a) 9.714 gm sodium acetate and 14.714 gm sodium barbitol in distilled water to make 500 ml.

(b) Four per cent osmium tetroxide, two gm in 50 ml distilled water.

(c) 0.1 N HCl

Preparation of fixative

Take 20 ml buffer solution and add 0.1 N HCl (20 ml approx.) to bring to pH 7.5, add distilled water to make the final volume 50 ml. Just before using mix this solution with an equal volume of the osmium tetroxide solution. 5.5 gm sucrose should be added to every 100 ml of fixative to raise the concentration to a molarity of 0.3 M.

II. Preparation of Araldite embedding medium

22 ml HY 964 hardener
22 ml CY 212 epoxy
12 drops DY 064 accelerator
20 drops di-butyl-phthalate

or alternatively:

18.6 gm HY 964 hardener
22.3 gm CY 212 epoxy
0.65 gm DY 064 accelerator
0.3 gm di-butyl-phthalate

The mixture should be stirred for ten minutes, bubbles may be removed by placing the fresh Araldite in an oven for a few minutes.

III. Lead citrate stain for electron microscopy

Lead citrate was prepared according to the method of Reynolds (1963) and also by dissolving lead citrate directly in sodium hydroxide.

1.33 gm lead nitrate, 1.76 gm sodium citrate and 30 ml of distilled water are placed in a volumetric flask and agitated for a few minutes. After half an hour 8 ml of 1N sodium hydroxide are added and the total volume is made up to 50 ml. The solution is now ready for use. By preparing lead citrate in this way contamination with lead carbonate is said to be reduced (Pease, 1964). Lead carbonate contamination of grids can be a serious problem producing a dense deposit on the surface of the grids. However, lead citrate stain can be made directly by dissolving 0.2 gm of lead citrate in 9.0 ml of distilled water and adding 1.0 ml of 10 N sodium hydroxide. The latter technique was normally used and Reynolds' method was only used when carbonate precipitate could not be eliminated.

IV. Staining of Araldite sections for light microscopy

Araldite sections for examination by light microscopy were stained with toluidine blue after the method of Trump, Smuckler and Benditt (1961).

Staining solution

1 per cent toluidine blue adjusted to pH 10 with borax/ boric acid buffer

Procedure

(a) Sections are transferred to a pool of distilled water on a glass microscope slide.

(b) The slide is placed on a hot plate to evaporate the water and dry sections onto the slide.

(c) Toluidine blue solution is pipetted on to the slide and heated at 50°C for 30 seconds.

(d) The slide is washed with distilled water and dried.

(e) The sections are mounted under a cover slip using Araldite or DePeX medium.

To prevent sections fading with time, the slide should be baked for 24 hours on a hotplate between the last two steps.

V. Holmes' silver staining technique

Solutions

(a) 20 per cent silver nitrate in distilled water

(b) Impregnating solution

27.5 ml boric acid solution

22.5 ml borax solution

300 ml distilled water

3 ml lutidine

0.5 ml 20 per cent silver nitrate

(c) Reducer

1 gm hydroquinone

10 gm sodium sulphate crystals

100 ml distilled water

(d) 0.2 per cent gold chloride for toning

(e) Two per cent oxalic acid

(f) 5 per cent sodium thiosulphate solution for fixing

Procedure

(a) Bring sections down through alcohols to water.

(b) Wash for ten minutes in running tapwater.

(c) Rinse in distilled water.

(d) Leave in 20 per cent silver nitrate in the dark for 16 hours.

(e) Wash for ten minutes in three changes of distilled water.

(f) Place slides in impregnating solution at 37°C for 24 hours.

(g) Remove superfluous fluid and place in fixer for two minutes.

(h) Wash in running tapwater for three minutes and rinse in distilled water.

(i) Tone in 0.2 per cent gold chloride for three minutes until sections are no longer brown.

(j) Rinse briefly in distilled water.

(k) Place in two per cent oxalic acid until axons are blue/black, approximately nine minutes.

(l) Rinse in distilled water.

(m) Fix with five per cent sodium thiosulphate for five minutes.

(n) Wash in tapwater for ten minutes.

(o) Counterstain if desired.

(p) Dehydrate, clear and mount.

VI. Physiological saline after Russell (1968)

(a) 6.76 gm NaCl

(b) 0.15 gm KCl

(c) 0.26 gm $CaCl_2$

(d) Distilled water to make one litre

APPENDIX II

AN ELEMENTARY MATHEMATICAL TREATMENT OF SURFACE WAVES

To render the subject of surface waves amenable to simple mathematical treatment, a simplified model of a real fluid must be used. The equation of motion thus obtained can then be applied to real fluids by introducing terms dependant on viscosity and surface tension to give a qualitative description of the phenomenon.

In deriving the simplified model, the following conditions apply:

(a) The real liquid is regarded as a *continuous* and *incompressible fluid*; ρ^* is therefore constant. In most cases under normal conditions, the compressibility of a liquid is negligible.

(b) The real liquid is regarded as *inviscid*, that is, the absolute viscosity $\mu = 0$. In reality, the effects of viscosity are only appreciable where velocity gradients are large such as at the boundaries of the liquid.

(c) The vorticity $\nabla \wedge \underset{\sim}{q} = 0$ (curl $\underset{\sim}{q} = 0$), the motion of the fluid being *irrotational*, with no vorticies or vortex lines.

That the curl of $\underset{\sim}{q}$ vanishes is sufficient condition for the existence of a scalar field function ϕ (the velocity potential) such that $\underset{\sim}{q} = -\nabla\phi$. The components of velocity along the x, y, and z directions can thus be written:

$$q_x = \frac{-\partial\phi}{\partial x}, \quad q_y = \frac{-\partial\phi}{\partial y}, \quad q_z = \frac{-\partial\phi}{\partial z}$$

---

*For table of mathematical symbols see page 197.

(d) The Continuity Equation, expressing the conservation of mass, gives the relationship (there being no sources or sinks)

$$\rho \nabla . \underline{q} + \frac{D\rho}{Dt} = 0 \quad \left(\rho \, \text{div} \, \underline{q} + \frac{D\rho}{Dt} = 0 \right)$$

which, applied to the model under consideration, reduces to

$$\nabla . \underline{q} = 0 \qquad \text{(i)}$$

since from (a) ρ = constant.

From (c), $\underline{q} = -\nabla\phi$ substituting this in (i) yields Laplace's Equation:-

$$\nabla^2 \phi = 0$$

or $$\frac{\partial \phi^2}{\partial x^2} + \frac{\partial^2 \phi}{\partial y^2} + \frac{\partial^2 \phi}{\partial z^2} = 0 \qquad \text{(ii)}$$

(e) The Equation of Motion for the fluid (Euler's Equation) is derived from Newton's law of motion:

Force = mass x acceleration

and can be written:

$$\underline{F} - \frac{1}{\rho} \nabla p = \frac{D\underline{q}}{Dt}$$

Evaluating this equation along any line in the fluid yields a more convenient form of the equation (Bernouilli's Equation) in which gravity has been substituted for the external body force:

$$\frac{p}{\rho} = \frac{\partial \phi}{\partial t} - gy - \frac{1}{2} q^2 \qquad \text{(iii)}$$

(f) At the fixed boudary of the fluid (in this case, the bottom of the sheet of water), the fluid velocity normal to the boundary must vanish, that is, the gradient of the velocity potential function ϕ normal to the boundary $= 0$ or

$$\frac{\partial \phi}{\partial n} = 0 \qquad \text{(iv)}$$

(g) The free surface of the fluid is going to be one of equi-pressure or constant pressure. Hence, at the surface:

$$\frac{Dp}{Dt} = 0$$

or expanding

$$\frac{\partial p}{\partial t} + q_x \frac{\partial p}{\partial x} + q_y \frac{\partial p}{\partial y} + q_z \frac{\partial p}{\partial z} = 0 \qquad \text{(v)}$$

(h) For the propagation of waves of height $\eta = \eta(x,t)$ above the mean level $(y=0)$, the equation of the free surface is given by:

$$y = \eta(x,t)$$

and since the surface moves with the fluid then

$$\frac{D}{Dt}\left[y-\eta(x,t)\right]=0$$

or

$$\frac{\partial \eta}{\partial t}+q_x\frac{\partial \eta}{\partial x}=q_y \qquad \text{(vi)}$$

Thus, the equations for ϕ and η describing the surface waves, must satisfy (i) to (v) of (a) to (g). Also in deriving the mathematical expressions describing the surface waves to be propagated, the following conditions will be imposed:

(A) The waves will be due to small oscillatory motions at or near the surface of the fluid. As such both squares of velocity terms, and the tangent of the wave form will be very small and can be neglected.

(B) The fluid surface will be an unlimited sheet.

(C) The depth of the fluid will be large compared with the wavelength of the waves - $h \gg \lambda$ or $\frac{h}{\lambda} \gg 1$.

(D) The discussion will be limited to the x and y coordinates of a right-hand coordinate system, in which the x axis is taken along the undisturbed surface and the y axis vertically upward.

Collecting together the equations to be satisfied and applying (A) - (D):

$$\frac{\partial^2 \phi}{\partial x^2} + \frac{\partial^2 \phi}{\partial y^2} = 0 \qquad \text{(ii)}$$

$$p = \rho \frac{\partial \phi}{\partial t} - \rho g y \qquad \text{(iii)}$$

$$\left.\frac{\partial \phi}{\partial y}\right|_{y=-h} = 0 \qquad \text{(iv)}$$

$$\frac{\partial p}{\partial t} + \frac{\partial p}{\partial x}\frac{\partial \phi}{\partial x} + \frac{\partial p}{\partial y}\frac{\partial \phi}{\partial y} = 0 \qquad \text{(v)}$$

$$\left.\frac{\partial \eta}{\partial t}\right|_{y=0} = -\left.\frac{\partial \phi}{\partial y}\right|_{y=0} \qquad \text{(vi)}$$

Differentiating (iii) with respect to time, x and y and substituting the expressions for $\frac{\partial p}{\partial t}$, $\frac{\partial p}{\partial x}$ and $\frac{\partial p}{\partial y}$ in (v) yields:

$$\left.\frac{\partial^2 \phi}{\partial t^2}\right|_{y=0} + g \left.\frac{\partial \phi}{\partial y}\right|_{y=0} = 0 \qquad \text{(vii)}$$

Assume a solution to (ii) $\phi = f(y) \cos(kx - \omega t)$

Substituting this in (ii):

$$\frac{\partial^2 f(y)}{\partial y^2} - k^2 f(y) = 0$$

and $f(y)$ is of the form

$$f(y) = A e^{ky} + B e^{-ky}$$

Using equation (iv) at the fixed boundary and condition (C) $\frac{h}{\lambda} \gg 1$, then $B = 0$ and ϕ becomes

$$\underline{\phi = A e^{ky} \cos(kx - \omega t)}$$

ϕ thus decays exponentially with depth, such that at depths of $\lambda/2$ and λ has values 4% and 0.2% respectively of its surface value. Hence, h need only be greater than λ to satisfy the boundary conditions.

From equation (vi) an expression can be derived via the velocity potential function ϕ for the displacement η. Thus:

$$\eta = -\int_t \left.\frac{\partial \phi}{\partial t}\right|_{y=0} dt$$

and $$\eta = \eta_0 \sin(kx - \omega t)$$

where $$\eta_0 = \frac{kA}{\omega}$$

so that the waves propagate along the positive x direction with amplitude η_0 and velocity $c = \frac{\omega}{k} = \sqrt{\frac{g}{k}}$ (using equation (vii)).

Under the conditions of the model liquid, the waves will propagate to infinity since no wave energy is being dissipated to overcome viscous forces.

The paths of the fluid particles can be obtained by noting that:

$$q_x = \frac{\partial x}{\partial t} = \frac{-\partial \phi}{\partial x} \quad \text{and} \quad q_y = \frac{\partial y}{\partial t} = \frac{-\partial \phi}{\partial y}$$

so that

$$x = -\int_t \frac{\partial \phi}{\partial x}\, \partial t \qquad \text{and} \qquad y = -\int_t \frac{\partial \phi}{\partial y}\, \partial t$$

Squaring and adding the expressions for x and y, yields

$$x^2 + y^2 = \eta_0^2\, e^{2ky}$$

and comparing with the standard form of a circle

$$x^2 + y^2 = (\text{radius})^2$$

gives

$$\underline{\text{radius} = \eta_0\, e^{ky}}$$

As expected from the expression for ϕ, the radius decays exponentially with depth so that $r_{\lambda/2} = 0.04\, r_{\text{surface}}$.

To make the above analysis of surface waves more applicable to real fluids (in this case water), the fluid is attributed a finite viscosity. As a result viscous forces (or "frictional forces") will now exist between adjacent layers of liquid. To overcome these forces, the wave will have to do work and this

is derived from its energy. In other words to maintain the motion, the energy of the wave will be dissipated.

For the above surface waves, the surface stresses due to the velocity components are given by:

$$\text{Normal component} = -p + 2\mu \frac{\partial q_y}{\partial y}$$

$$\text{Tangential component} = \mu \left(\frac{\partial q_y}{\partial x} + \frac{\partial q_x}{\partial y} \right)$$

Substituting the expressions for $\frac{\partial q_y}{\partial x}$, $\frac{\partial q_y}{\partial y}$ and $\frac{\partial q_x}{\partial y}$ derived from (c) and the expression for ϕ, the stress components become:

$$\text{Normal component} = -p - 2\mu k\omega \eta_0 \cos(kx - \omega t)$$

$$\text{Tangential component} = 2\mu k\omega \eta_0 \sin(kx - \omega t)$$

The total rate of doing work against these forces or Power output is:

$$P = 2\mu k\omega \eta_0 q_x \sin(kx - \omega t) - q_y \left[p + 2\mu k\omega \eta_0 \cos(kx - \omega t) \right]$$

(since Power $= \frac{d}{dt}$ (Work) = Force x velocity)

Substituting for q_x and q_y, and taking the mean over one cycle by integrating, the mean power output is:

$$\overline{P} = 2\mu k\omega^2 \eta_0$$

which must equal the rate of dissipation of energy given by:

$$\frac{d}{dt}\left(\frac{1}{2}\eta_o^2 k c^2 \rho\right)$$

where the term in parenthesis is the total energy of the wave per unit surface area. Hence:

$$\frac{d}{dt}\left(\frac{1}{2}\eta_o^2 k c^2 \rho\right) = -2\mu k \omega^2 \eta_o^2$$

and

$$\frac{d\eta_o}{dt} = -2\nu k^2 \eta_o$$

which has the solution

$$\eta_o = \eta_{in} e^{-2\nu k^2 t}$$

Thus for a viscous fluid

$$\eta = \eta_{in} e^{-2\nu k^2 t} \sin(kx - \omega t)$$

so that the initial amplitude of the wave decreases with distance from the origin of disturbance. The radii of the particle paths, by direct substitution for η_o are given by

$$r = \eta_{in} e^{-2\nu k^2 t} e^{ky}$$

Figures 72 and 73 show the relationships existing between displacement and distance from origin, radius and depth, and radius and distance (for a depth of $\pi/4$) for surface waves in water of $\lambda = 1$ cm and $\lambda = 2$ cms, and initial displacements of $\eta_{in} = 1$ mm, 1.5mm and 2mm.

It is evident that motions derived from small perturbations of the liquid surface persist at enormously greater distances from the disturbance along the surface than they do with depth below the surface. The discussion of surface waves has been derived from Milne-Thomson (1949), Rutherford (1959), and Ramsey (1960).

TABLE OF MATHEMATICAL SYMBOLS

ρ = density

μ = absolute viscosity

ν = kinematic viscosity = $\frac{\mu}{\rho}$ (for water $\nu = 0.01\ cm^2\ sec^{-1}$)

p = pressure

g = acceleration due to gravity

q = velocity

$q_x\ q_y\ q_z$ = velocity components along x, y, z axes

ϕ = velocity potential function

$\underline{F}$ = body force per unit mass of fluid

η = displacement of curve above undisturbed surface of fluid

η_o = amplitude of wave displacement

η_{in} = amplitude at origin (for case of viscous fluid)

k = propagation constant = $\frac{2\pi}{\lambda}$

λ = wavelength of surface wave

ω = angular frequency

c = velocity of propagation of wave = $\frac{\omega}{k}$

h = depth of fluid

t = time

r = radius of fluid particle path

P = Power

$\bar{P}$ = Mean Power per cycle

$\frac{D}{Dt}$ = rate of change moving with the fluid

$\left.\begin{matrix} A \\ B \end{matrix}\right\}$ = constants of integration

$\nabla_\wedge$ = curl

$\nabla\phi$ = grad

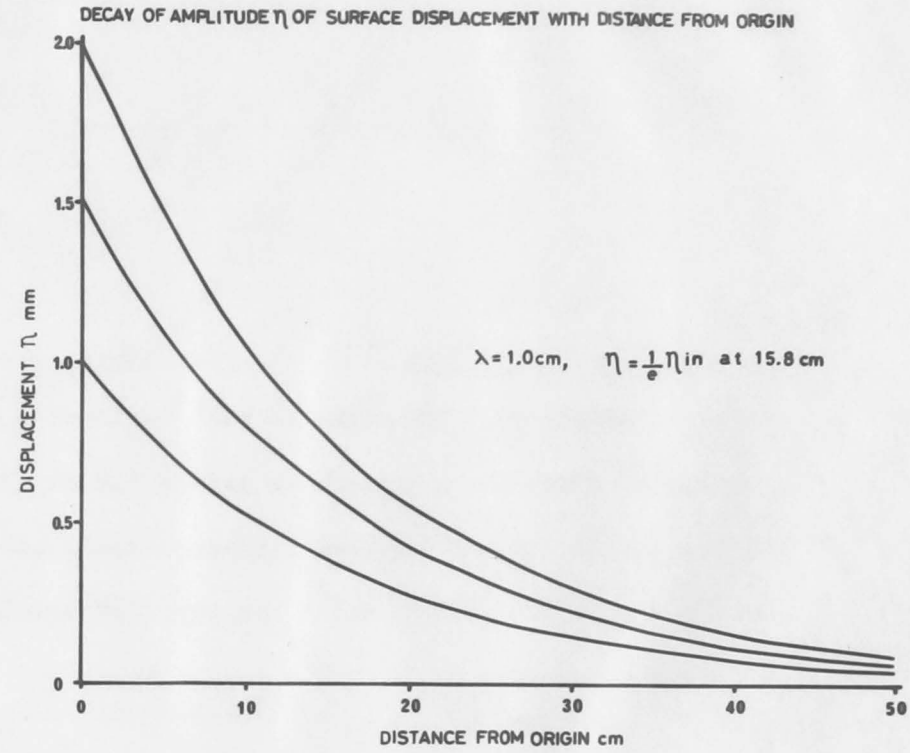
$\frac{\partial}{\partial a}$ = partial derivative with respect to a

Figure 72

(A) This graph shows the decay of surface wave amplitude with distance from the origin. Three initial values of displacement (η_{in}) are considered: 1.0 mm, 1.5 mm and 2.0 mm. The wavelength λ is 1.0 cm for each case.

(B) This graph shows the effect of an increased wavelength on the decay rate of surface wave amplitude. Initial displacements are as before; the wavelength λ is 2.0 cm for each case.

A


DECAY OF AMPLITUDE η OF SURFACE DISPLACEMENT WITH DISTANCE FROM ORIGIN
DISPLACEMENT η mm
2.0
1.5
1.0
0.5
0
λ = 1.0 cm, η = 1/e η in at 15.8 cm
0
10
20
30
40
50
DISTANCE FROM ORIGIN cm

B

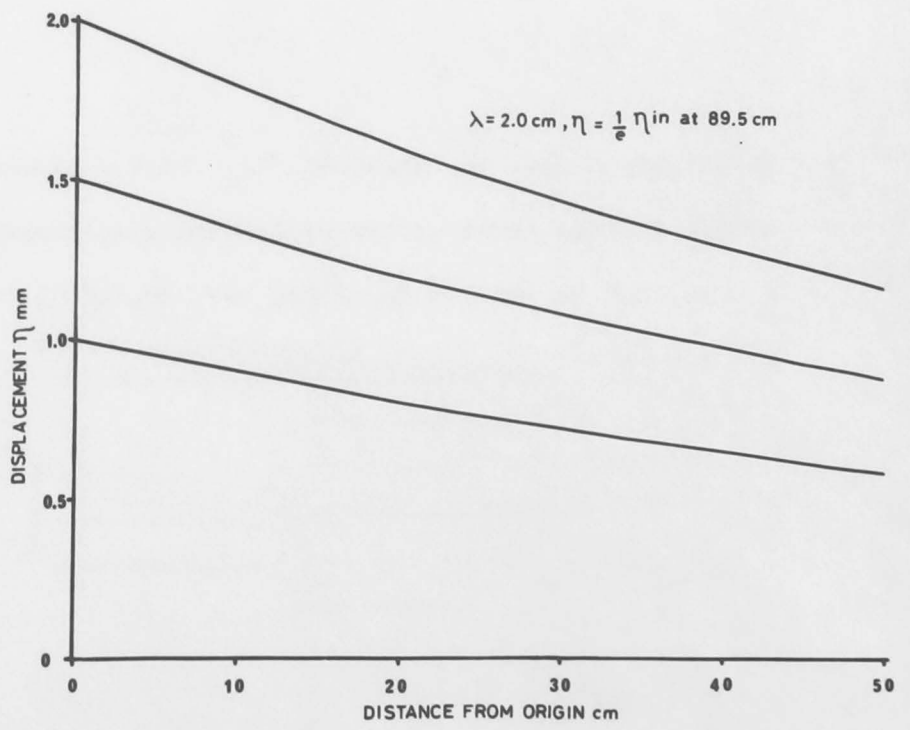

DISPLACEMENT η mm
2.0
1.5
1.0
0.5
0
λ = 2.0 cm , η = 1/e η in at 89.5 cm
0
10
20
30
40
50
DISTANCE FROM ORIGIN cm

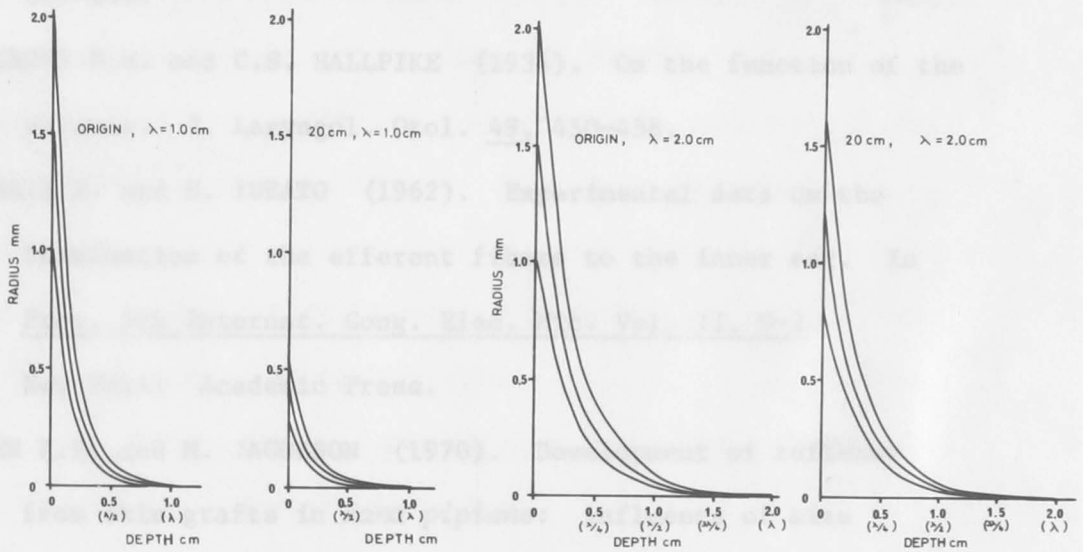
Figure 73

(A) Graphs showing decay of particle path radii with depth. The initial displacements are as before 1.0 mm (lower line), 1.5 mm and 2.0 mm (upper line). Radii are considered at the origin and 20 cm away for wavelengths 1.0 cm and 2.0 cm.

(B) These graphs show the decay of $r_{\lambda/4}$ with distance from origin for the three values of initial displacement 1.0 mm, 1.5 mm and 2.0 mm having wavelengths 1.0 cm and 2.0 cm.

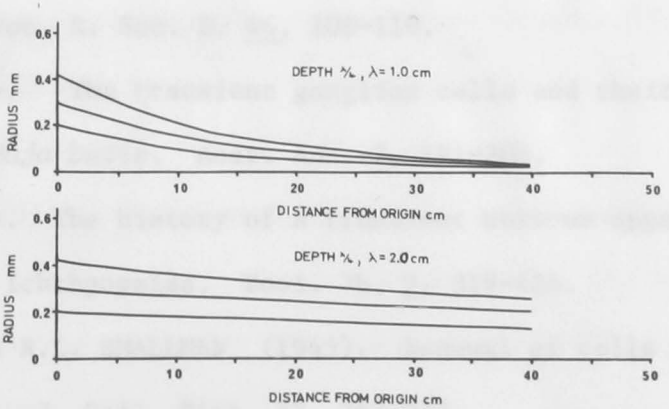
A

DECAY OF PARTICLE PATH RADII WITH DEPTH AT ORIGIN AND 20 cm FROM ORIGIN



B

DECAY OF PARTICLE PATH RADII, AT DEPTH λ/4, WITH DISTANCE FROM ORIGIN



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